National Library of Canada

Acquisitions and Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada

Direction des acquisitions et des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

## NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

## AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

# MORPHOLOGICAL CHARACTERISTICS OF DAMAGED AND REPAIRED LINGUAL NERVES IN CAT

©

BY

DANIELLA COELHO DE ANDRADE

A thesis submitted to the Faculty of Graduate
Studies and Research in partial fulfilment of the
requirements for the degree of MASTER OF SCIENCE

IN ORAL BIOLOGY
FACULTY OF DENTISTRY

Edmonton, Alberta
SPRING, 1993

National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-82063-2

Canada

# UNIVERSITY OF ALBERTA

## RELEASE FORM

NAME OF AUTHOR: DANIELLA COELHO DE ANDRADE

TITLE OF THESIS: MORPHOLOGICAL CHARACTERISTICS OF DAMAGED AND REPAIRED LINGUAL NERVES IN CAT

DEGREE: MASTER OF SCIENCE

YEAR THIS DEGREE GRANTED: 1993

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

Daniella Coelho de Andrade

Rua Tomé de Souza, 395/701
Belo Horizonte - M.G.
Brasil - 30140

Date: March 22, 1993

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled MORPHOLOGICAL CHARACTERISTICS OF DAMAGED AND REPAIRED LINGUAL NERVES submitted by DANIELLA COELHO DE ANDRADE in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE.

Dr. G.R. Holland

Nadine Milos

Dr. N. C. Milos

Dr. L. W. Kline

B. K. Arora

Dr. B. K. Arora

Date: March 22, 1993

# ABSTRACT

In this study transected lingual nerves 3 and 6 months after injury with and without repair were investigated to establish whether or not the morphological recovery of transected lingual nerves is improved by epineurial suturing. In one group (8 cats), the left lingual nerves were transected 4mm distal to the lingual-chorda junction and prepared for histology 3 months (3 cats) or 6 months (5 cats) later. In another group (6 cats) sutures were placed in the epineurium immediately after transection. The right lingual nerves served as controls. Proximal to the injury it was possible to recognize the chorda tympani and lingual nerves as discrete nerve trunks in all groups except that which was left unrepaired for 3 months. Distal to the injury this was possible only in one animal 6 months after transection. Proximal to the lesion a substantial increase in the number of unmyelinated axons and a decrease in the number of myelinated was observed in the group with no repair and a shorter time recovery. The repaired group had 14% myelinated counts increase. In all 3 groups the number of myelinated axons distal to the injury was reduced but markedly only in the groups that were not repaired. The thickness of the myelin sheath and the circumference of the axons decreased in the groups which had a shorter recovery time. There was an increase in the number of unmyelinated axons distal to the injury in the groups which had a shorter recovery time. These findings suggest that both the repair technique used and the

time allowed for recovery have an effect on the outcome of regeneration.

# ACKNOWLEDGEMENTS

I would like to acknowledge the support given by a number of people who helped me througout my program at this university to complete this work.

My supervisor, Dr. Rex Holland, for all his assistance, encouragement, participation and friendly guidance towards the completion of this project.

My committee members, Dr. N.C. Milos, Dr. L.W. Kline and Dr. B. K. Arora for their kindness in assisting and providing thoughtful comments to improve this thesis.

Mrs. Enid Pehowich for her great support and valuable participation throughout the completion of the data collection.

Dr. Ming Cheng for his kind orientation and valuable assistance through the data collection.

## TABLE OF CONTENTS

CHAPTER ONE: INTRODUCTION

INTRODUCTION.....1

STATEMENT OF THE PROBLEM.....3

OBSERVATION AND RESEARCH HYPOTHESES.....5

DELIMITATIONS AND LIMITATIONS.....7

DEFINITION OF TERMS.....8

CHAPTER TWO: REVIEW OF THE LITERATURE

LINGUAL AND CHORDA TYMPANI NERVE CHARACTERISTICS

ANATOMICAL PATHWAY OF THE HUMAN LINGUAL NERVE.....10

CLASSIFICATION OF FIBRES.....11

STRUCTURE AND COMPOSITION OF THE LINGUAL NERVE.....12

CONNECTIVE TISSUE PROTECTING THE NERVE TRUNK.....15

SUNDERLAND'S CLASSIFICATION OF INJURIES.....16

ETIOLOGY OF LINGUAL NERVE DAMAGE.....18

INCIDENCE OF LINGUAL NERVE DAMAGE.....22

CLINICAL CONSEQUENCES OF LINGUAL NERVE INJURY.....24

FACTORS AFFECTING THE NERVE RECOVERY.....26

MORPHOLOGICAL CONSEQUENCES OF PERIPHERAL NERVE DAMAGE

INTRODUCTION.....28

AXON POPULATION AROUND INJURY SITES.....29

MYELINATED AXONAL POPULATION CENTRAL TO DAMAGE.....30

NUMBER OF UNMYELINATED AXONS PROXIMAL TO DAMAGE.....32

CELL BODY COUNTS.....34

NUMBER OF MYELINATED AXONS DISTAL TO DAMAGE.......35
NUMBER OF UNMYELINATED AXONS DISTAL TO DAMAGE.....37
AXON SIZE AND MYELIN THICKNESS PROXIMAL AND DISTAL TO DAMAGE..............................38
SCHWANN CELL UNIT PROXIMAL AND DISTAL TO DAMAGE...42

NERVE REPAIR

INTRODUCTION...................................43
FACTORS ACTING PROXIMAL AND DISTAL TO THE SUTURE LINE....................................44
METHODS OF NERVE REPAIR........................48
NEUROLYSIS.....................................49
EPINEURAL REPAIR...............................49
GROUP FASCICULAR REPAIR........................51
FASCICULAR REPAIR..............................51
NERVE RECONNECTION.............................52
NERVE GRAFTING.................................52
TUBULATION.....................................53

CHAPTER THREE: METHODOLOGY

SUBJECTS AND EXPERIMENTAL INJURY...................55
MORPHOMETRICAL PROCEDURES..........................60
VALIDATION OF THE METHODS..........................66
DATA COLLECTION....................................72
ANALYSIS OF DATA...................................73

CHAPTER FOUR: RESULTS

INTRODUCTION....................................75

NUMBER OF MYELINATED AXONS......................75

GROUP I.........................................80

GROUP II........................................83

GROUP III.......................................86

NUMBER OF UNMYELINATED AXONS....................93

GROUP I.........................................93

GROUP II........................................96

GROUP III.......................................99

MYELINATED AXONAL SIZE..........................106

GROUP I.........................................106

GROUP II........................................111

GROUP III.......................................112

THICKNESS OF THE MYELIN SHEATH..................128

GROUP I ........................................128

GROUP II........................................131

GROUP III.......................................134

REMAK BUNDLES...................................140

GROUP I.........................................140

GROUP II........................................143

GROUP III.......................................146

CHAPTER FIVE: DISCUSSION

INTRODUCTION....................................152

NUMBER OF MYELINATED AXONS PROXIMAL TO THE INJURY.....153

TOTAL MYELINATED COUNTS.........................153

LINGUAL AND CHORDA TYMPANI COUNTS.....................154
NUMBER OF MYELINATED AXONS DISTAL TO THE INJURY.......156
TOTAL NUMBER OF UNMYELINATED AXONS PROXIMAL TO THE INJURY ..................................................158
TOTAL COUNTS.........................................158
LINGUAL AND CHORDA TYMPANI COUNTS.....................159
NUMBER OF UNMYELINATED AXONS DISTAL TO THE INJURY.....160
MYELINATED AXONAL SIZE PROXIMAL TO THE INJURY.........161
MYELINATED AXONAL SIZE DISTAL TO THE INJURY...........163
THICKNESS OF THE MYELIN SHEATH PROXIMAL TO THE INJURY.164
THICKNESS OF THE MYELIN SHEATH DISTAL TO THE INJURY...165
REMAK BUNDLES PROXIMAL AND DISTAL TO THE INJURY.......166

CHAPTER SIX: CONCLUSIONS

INTRODUCTION..........................................168
SUMMARY OF THE HYPOTHESES STUDIED.....................170
CONCLUSIONS...........................................172
REFERENCES............................................174

## LIST OF TABLES

TABLE 1. CAUSES OF LINGUAL NERVE INJURY................20

TABLE 2. NUMBER AND SIZE OF MYELINATED AXONS FOR LIGHT AND ELECTRON MICROSCOPE......................71

TABLE 3. NUMBER OF MYELINATED AXONS FOR GROUP I.........81

TABLE 4. STATISTICS OF MYELINATED AXONS FOR GROUP I.....82

TABLE 5. NUMBER OF MYELINATED AXONS FOR GROUP II........84

TABLE 6. STATISTICS OF MYELINATED AXONS FOR GROUP II....85

TABLE 7. NUMBER OF MYELINATED AXONS FOR GROUP III.......88

TABLE 8. STATISTICS OF MYELINATED AXONS FOR GROUP III...89

TABLE 9. NUMBER OF UNMYELINATED AXONS FOR GROUP I.......94

TABLE 10. STATISTICS OF UNMYELINATED AXONS FOR GROUP I...95

TABLE 11. NUMBER OF UNMYELINATED AXONS FOR GROUP II.....97

TABLE 12. STATISTICS OF UNMYELINATED AXONS FOR GROUP II..98

TABLE 13. NUMBER OF UNMYELINATED AXONS FOR GROUP III....100

TABLE 14. STATISTICS OF UNMYELINATED AXONS FOR G-III....102

TABLE 15. MYELINATED AXONAL SIZE FOR GROUP I............109

TABLE 16. STATISTICS OF MYELINATED AXONAL SIZE FOR GROUP I...................................110

TABLE 17. MYELINATED AXONAL SIZE FOR GROUP II..........116

TABLE 18. STATISTICS OF MYELINATED AXONAL SIZE FOR GROUP II..................................117

TABLE 19. MYELINATED AXONAL SIZE FOR GROUP III.........123

TABLE 20. STATISTICS OF MYELINATED AXONAL SIZE FOR GROUP III.................................124

TABLE 21. THICKNESS OF THE MYELIN SHEATH FOR GROUP I....129

TABLE 22. STATISTICS OF THE THICKNESS OF THE MYELIN SHEATH FOR GROUP I..............................130
TABLE 23. THICKNESS OF THE MYELIN SHEATH FOR GROUP II....132
TABLE 24. STATISTICS OF THE THICKNESS OF THE MYELIN SHEATH FOR GROUP II...........................133
TABLE 25. THICKNESS OF THE MYELIN SHEATH FOR GROUP III .135
TABLE 26. STATISTICS OF THE THICKNESS OF THE MYELIN SHEATH FOR GROUP III..........................136
TABLE 27. REMAK BUNDLES FOR GROUP I.....................141
TABLE 28. STATISTICS OF REMAK BUNDLES FOR GROUP I.......142
TABLE 29. REMAK BUNDLES FOR GROUP II....................144
TABLE 30. STATISTICS OF REMAK BUNDLES FOR GROUP II......145
TABLE 31. REMAK  BUNDLES FOR GROUP III..................147
TABLE 32. STATISTICS OF REMAK BUNDLES FOR GROUP III.....148

## LIST OF FIGURES

FIGURE 1. SITE OF INJURY....56

FIGURE 2. EPINEURAL SUTURING....58

FIGURE 3. PROCESSED NERVE SEGMENTS....62

FIGURE 4. ANALYZED SITES....63

FIGURE 5. SAMPLING TECHNIQUES....64

FIGURE 6. SQUARE SAMPLING TECHNIQUES....65

FIGURE 7. SYSTEMATIC SECTOR SAMPLING TECHNIQUE....68

FIGURE 8. SYSTEMATIC SQUARE SAMPLING TECHNIQUE....69

FIGURE 9. LINGUAL NERVE LIGHT MICROGRAPHS CONTROL AND PROXIMAL SECTIONS....77

FIGURE 10. LINGUAL NERVE LIGHT MICROGRAPH DISTAL TO THE TRANSECTION....78

FIGURE 11. LINGUAL AND CHORDA TYMPANI DISTAL SECTION....79

FIGURE 12. LINGUAL ELECTRON MICROGRAPHS CONTROL AND PROXIMAL SECTION FOR GROUP I....107

FIGURE 13. LINGUAL NERVE ELECTRON MICROGRAPH DISTAL TO THE TRANSECTION FOR GROUP I....108

FIGURE 14. LINGUAL ELECTRON MICROGRAPHS CONTROL AND PROXIMAL SECTIONS FOR GROUP II....113

FIGURE 15. CHORDA TYMPANI ELECTRON MICROGRAPHS DISTAL TO THE TRANSECTION FOR GROUP II....114

FIGURE 16. LINGUAL ELECTRON MICROGRAPH DISTAL TO THE TRANSECTION FOR GROUP II....115

FIGURE 17. LINGUAL ELECTRON MICROGRAPHS CONTROL AND PROXIMAL SECTION FOR GROUP III....120

FIGURE 18. CHORDA TYMPANI ELECTRON MICROGRAPHS DISTAL TO THE TRANSECTION FOR GROUP III..............121
FIGURE 19. LINGUAL ELECTRON MICROGRAPH DISTAL TO THE TRANSECTION FOR GROUP III.....................122

# LIST OF GRAPHICS

GRAPHIC 1. TOTAL MYELINATED AXON COUNTS..............90

GRAPHIC 2. LINGUAL MYELINATED AXON COUNTS...........91

GRAPHIC 3. CHORDA TYMPANI MYELINATED AXON COUNTS.....92

GRAPHIC 4. TOTAL UNMYELINATED AXON COUNTS...........103

GRAPHIC 5. LINGUAL MYELINATED AXON COUNTS...........104

GRAPHIC 6. CHORDA TYMPANI MYELINATED AXON COUNTS....105

GRAPHIC 7. TOTAL MYELINATED AXONAL SIZE............125

GRAPHIC 8. LINGUAL MYELINATED AXONAL SIZE...........126

GRAPHIC 9. CHORDA TYMPANI MYELINATED AXONAL SIZE....127

GRAPHIC 10. TOTAL THICKNESS OF THE MYELIN SHEATH.....137

GRAPHIC 11. LINGUAL THICKNESS OF THE MYELIN SHEATH...138

GRAPHIC 12. CHORDA TYMPANI THICKNESS OF THE MYELIN SHEATH ........................................139

GRAPHIC 13. TOTAL REMAK BUNDLES......................149

GRAPHIC 14. LINGUAL REMAK BUNDLES....................150

GRAPHIC 15. CHORDA TYMPANI REMAK BUNDLES.............151

# CHAPTER ONE

# INTRODUCTION

## INTRODUCTION

Standard texts of oral surgery describe the occurrence and incidence of inferior alveolar nerve damage during lower third molar surgery (e.g., Howe, 1974). However, few comment on the effects of surgery on the lingual nerve. Those that do so (e.g., Kruger, 1981) suggest that the risk is due to the proximity of the lingual nerve to the operation site. They also suggest that the damage, when it occurs, is likely to be the result of the careless use of surgical instruments (e.g., Howe, 1974).

Recent surveys show that the lingual nerve is frequently damaged during the removal of lower third molars (Mason, 1988) and may be more susceptible to damage than the inferior alveolar (Blackburn and Brammley, 1989). Initial sensory alteration within the distribution of the lingual nerve following mandibular third molar extraction has been variously reported to be 1% (Rud, 1970), 6.6% (Rood, 1983), 11.5% (Mason, 1988), 11% (Blackburn and Brammley, 1989) and 22% (Von Arx and Simpson, 1989). Most of the patients showed full recovery within a few months but, in some cases (0.6%) (Mason, 1988), (0.5%) (Blackburn and Brammley, 1989), sensory deficits persisted. The large variation in the reported incidence of lingual nerve injury might be explained by differences in the surgical techniques used, differences in the interval between extraction and the assessment of sensory alteration and by differences in the kind of sensory tests used to assess the severity of injury (Robinson, 1992).

Other surgical techniques such as the modified distolingual splitting technique, in which the lingual soft tissue is not separated from bone, have been developed to decrease these potential complications (Davis et al., 1983). Even with these improvements, lingual nerve damage still occurred in 4.3% of 598 cases (Hochwald et al., 1983).

Attempts in repairing the damaged lingual nerves have led to satisfactory results in patients with persistent paraesthesia (Mozsary et al., 1984). Several techniques have been used but their efficacy has not been fully examined experimentally.

## STATEMENT OF THE PROBLEM

The morphological alterations following experimental damage have been reported for a number of nerves (Gutmann and Sanders, 1943; Cragg and Thomas, 1961; Aitken and Thomas, 1962; Horch and Lisney, 1981; Waite, 1984; Holland and Robinson, 1990-a). Robinson (1989) has reported electrophysiological changes after lingual nerve damage. Smith and Robinson (personal communication) have followed this with similar studies on repaired lingual nerves. However, little data is currently available on the morphological changes following lingual nerve damage and repair.

Holland and Robinson (1990-a) analyzed qualitatively and quantitatively the recovery of the inferior alveolar nerve after damage. They showed that complete recovery of the inferior alveolar nerve occurs after simple transection and suggested that this is related to the support provided by a bony canal resulting in good reapposition of the cut ends. After inferior alveolar transection the nerve ends remain in apposition, while after lingual nerve transection they may retract. The lingual nerve differs in structure and composition from the inferior alveolar and does not run inside a bony canal. After crushing injuries lingual nerve activity is almost normal, but it is significantly reduced after nerve transection (Robinson, 1989). Surgical repair of transected lingual nerves can improve the degree of recovery (Smith and Robinson, personal communication). The purpose of this investigation was an attempt to find out whether the morphological recovery of transected lingual nerves is improved by immediate repair using epineurial suturing.

## OBSERVATIONS AND RESEARCH HYPOTHESES

This study compared three groups of lingual nerves

I- Transected and allowed to recover for three months with no attempt to repair.

II- Transected and allowed to recover for six months with no attempt to repair.

III- Transected and allowed to recover for three months after immediate repair with epineurial sutures.

The following parameters were measured for each nerve examined.

1- Number, size distribution and sheath thickness of myelinated axons.

2- Number of unmyelinated axons and number of unmyelinated axons per Schwann cell.

The following nerve sites were examined.

1- Operated side proximal to the site of damage.

2- Operated side distal to the site of damage.

3- Unoperated side at a point similar to that of transection in the operated side.

The following hypothesis was tested:

That the morphological parameters cited above do not differ for;

I- Sites proximal to damage, both repaired and unrepaired

Versus unoperated side in the same group.

II- Sites distal to damage both repaired and unrepaired versus the unoperated side in the same group.

III- Sites distal to damage, both repaired and unrepaired versus proximal sites in the same group.

IV- Unrepaired (three months) versus unrepaired (six months).

V- Unrepaired (three months) versus repaired (three months).

## DELIMITATIONS

This study will be restricted to:

1 The use of an animal model.

2 One type of injury; that caused by transection of the lingual nerve with scissors.

3 The influence of epineural suturing immediately after transection on the success of nerve regeneration.

## LIMITATIONS

The limitations of this study included:

1 The use of histological techniques to assess the nerve recovery.

2 The use of the systematic random square sampling technique to estimate nerve fibre number and size in the nerve trunk.

## DEFINITIONS OF TERMS

### I- Neuroma

A neuroma is a disorganized mass of nerve and connective tissue that may form at the site of damage after peripheral nerve injury. Neuromas may be three or more times as large as the diameter of the normal nerve. Within the neuroma axons branch extensively and are incompletely ensheathed (Gutmann and Medawar, 1942-b; Edds, 1945). Unsheathed axons may be in close apposition.

### II- Schwann cell-unit

Unmyelinated axons in peripheral axons are ensheathed by Schwann cells. Each Schwann cell unit, or Remak Bundle, contains from one to several unmyelinated axons (Sunderland, 1978).

### III- Bands of Büngner

Bands of Büngner are the Schwann cells of the nerve distal to the site of injury that, because of Wallerian degeneration (degeneration that occurs distal to the site of damage characterized by break down of axons and myelin sheath), now lack axons and form clusters of aligned cells providing a potential pathway for regeneration (Weinberg and Spencer, 1978).

## IV- Endoneurial tube

Also called endoneurial sheath or Schwann tube, it is composed of the Schwann cells and collagen fibres that are packed around each nerve fibre (Holmes and Young, 1942).

# CHAPTER TWO

# REVIEW OF THE LITERATURE

## LINGUAL AND CHORDA TYMPANI NERVE CHARACTERISTICS

### ANATOMICAL PATHWAY OF THE HUMAN LINGUAL NERVE

The lingual nerve in human has a consistent pathway (Rezai and Bayley, 1988). It arises from the posterior division of the mandibular division of the trigeminal nerve within the infratemporal fossa. Deep to the inferior border of the lateral pterygoid muscle, the chorda tympani, a branch of the facial nerve joins the posterior aspect of the lingual nerve. The combined nerve courses down and forward between the mandible and medial pterygoid muscle to the submandibular region. At the upper mylohyoid line, the lingual nerve curves sharply anteriorly and continues horizontally on the superior aspect of the mylohyoid muscle into the oral cavity. At this point, the nerve is close to the submandibular gland and gives fibres to the submandibular ganglion. The lingual nerve then continues anteriorly close to the sublingual gland and after several divisions enters the tongue.

Recent studies have shown not only gross anatomical variations (Mozsary and Middleton, 1984), but also differences in the diameter and shape of the lingual nerve (Kiesselbach and Chamberlain, 1984). Kiesselbach and Chamberlain (1984), in dissection studies, demonstrated that the vertical position of the lingual nerve in the third molar area ranges from 2mm above to 7mm below the alveolar crest and its horizontal distance ranges from 0 to 3mm from the lingual plate. In 17.6% of the specimens the lingual nerves were at the same level or higher than the alveolar crest, and in 62% of the specimens the nerve was in contact with the lingual plate. In another dissection study (Loughner et al., 1990), the lingual nerve was passing through the medial portion of the lower belly of the lateral pterygoid muscle in about 6% of the specimens analyzed. At this point, nerve compression may occur if there is hypertrophy or spasm of the lateral pterygoid muscle. The chorda tympani was not found entrapped in the muscle suggesting that taste sensation would not be lost by lingual nerve entrapment in the lateral pterygoid muscle.

Kiesselbach and Chamberlain (1984) measured the lingual nerve at the third molar region. The lingual nerve had an average diameter of 1.86mm. Three nerves out of thirty-four cadaver heads had diameters of 0.5mm that represent the same diameter of a 25-gauge anaesthetic needle. In that region, 61.7% of lingual nerves were round, 17.6% were oval, and 20.5% were flat.

## CLASSIFICATION OF FIBRES

Nerve fibres are divided into three main groups (A, B and C) according to the characteristics of the nerve impulse and fibre size (Erlanger and Gasser, 1937). Group A represent the large fibres that carry the fastest conduction velocities consisting of myelinated somatic afferent and efferent fibres. It is subdivided according to the size of fibres into three other subgroups (A α, A β and A γ). A α fibres are the largest fibres of this subgroup showing diameters ranging from 15 to 20 µm and corresponding to efferent motor fibres. A β fibres have diameters ranging from 8 to 15 µm and are related to touch sensation, while A γ are the smallest fibres of these subgroups showing diameters between 2 and 5 µm, they have been associated with sharp, pricking pain and temperature. Group B consists of myelinated autonomic preganglionic fibres, whereas Group C consists of all the unmyelinated axons of autonomic and sensory nerves. The sensory impulses carried by C fibres are associated with a burning type of pain (Erlanger and Gasser, 1937).

## STRUCTURE AND COMPOSITION OF THE LINGUAL NERVE

Centrally to the lingual-chorda junction the lingual and the chorda tympani nerves in cats (Holland and Robinson, 1992) and in human (Girod et al., 1989) are monofascicular. The lingual nerve has an average of 3227 myelinated axons and 2472 unmyelinated axons central to the lingual-chorda junction in cats (Holland and Robinson, 1992). Another report shows an average of 4162 myelinated axons in cat (Biedenbach et al., 1975). The myelinated axon size distribution is unimodal with a predominance of small axons ranging from 2 to 6 µm in diameter. The largest axons in the lingual nerve are 19 µm in diameter and most of the myelinated axons are smaller than 9 µm (Biedenbach et al., 1975). More distally the cat lingual nerve is also monofascicular with an average of 3044 myelinated fibres and 2157 unmyelinated axons (Holland and Robinson, 1992).

Seventy-four per cent of myelinated axons present in the lingual nerve are within A γ size range axons and 26% within the A β range (Biedenbach, 1975). The lingual nerve axons also have thicker myelin sheaths than the chorda tympani axons. Axonal variations between cats, left and right sides as well as between points centrally and distally located relative to the lingual-chorda junction are small (Holland and Robinson, 1992).

The chorda tympani myelinated axons have more A γ axons (93%) than the lingual nerve (Holland and Robinson, 1992) representing the gustatory, thermosensitive and the preganglionic parasympathetic fibres (Foley, 1945). The chorda tympani has an average of 1322 myelinated and 779 unmyelinated axons central to the lingual-chorda junction and an average of 1390 myelinated and 856 unmyelinated axons distal to the lingual-chorda junction (Holland and Robinson, 1992).

Girod et al. (1989) investigated the fascicular structure of lingual and chorda tympani nerves in human cadavers. In nine of ten cases the chorda was monofascicular before its fusion with the lingual nerve. At this point and at the middle region between the lingual-chorda junction and the submandibular ganglion, the lingual nerve was either monofascicular or oligofascicular (two to four fascicles). At a more distal point, the lingual nerve is oligo or polyfascicular. The mean distance that the chorda tympani fascicle could be traced was 1.8 mm. In one case it could be traced for more than 5.2 mm distal to the lingual-chorda junction. In the third molar region, the chorda tympani axons could no longer be identified separately from the axons of the lingual nerve.

The lingual nerve carries mechanosensitive and thermosensitive afferent fibres from the tongue, as well as vasomotor efferent fibres to the tongue. The chorda tympani carries mechanosensitive and thermosensitive afferent fibres from the tongue, vasomotor efferent fibres to the tongue, secretomotor efferent fibres to the submandibular and sublingual glands, also the special sensory fibres for taste (Foley, 1945; Biedenbach et al., 1975). In addition to the preganglionic parasympathetic fibres, the chorda tympani also contains postganglionic fibres that, after synapsing in the submandibular ganglion, re-enter the nerve to be distributed to the tongue, sublingual gland and minor salivary glands in the floor of the mouth (Hiatt and Gartner, 1982). The combined nerve trunk supplies the anterior two-thirds of the tongue, the mucous membrane and gum on the lingual aspect of the mandible (Hiatt and Gartner, 1982). The large fibres present in the lingual nerve possibly are proprioceptive (Dubner, 1978), whereas the small myelinated and unmyelinated fibres are associated with autonomic activities, pain and temperature perception (Robinson, 1989). The chorda tympani carries impulses to both hot and cold stimuli, while the lingual fibres apparent respond only to cold (Robinson, 1992).

## CONNECTIVE TISSUE PROTECTING THE NERVE TRUNK

All peripheral nerves are protected by connective tissue. Many nerves, including the lingual, can consist of more than one fascicle (Girod et al., 1989). Between the fascicles and encircling the whole group is the epineurium, a highly vascularized dense, layered connective tissue that gives the peripheral nerve its tensile strength and much of its mechanical protection. Perineurium surrounds each individual fascicle, protecting them but allowing them to move independently. The third level of connective tissue, the endoneurium, is found within each individual fascicle, sometimes segmenting it. The endoneurium is largely concerned with nutritional support and adds little to mechanical protection of the nerve (Lundborg, 1988).

## SUNDERLAND'S CLASSIFICATION OF INJURIES

Nerve injuries are classified in several different ways depending on the morphological and functional alterations of the nerve fibres. Sunderland (1951) classified nerve injuries depending upon the type of nerve trunk structure affected after peripheral nerve injury. His classification is widely accepted and emphasises the importance of the connective tissue within the nerve trunk.

TYPE I - Also called neuropraxia, corresponds to a local demyelinating process followed by a conduction block in which the continuity or the nerve structures and the axons are preserved. It can be caused by light compression, which may occur if a retractor, placed between the lingual bone and the lingual mucoperiosteal flap, compresses the lingual nerve (Rood, 1983). Such a conduction block remains until the Schwann cells repair the local myelin, thus restoring the excitability of the cell. The time required for this process to happen is usually short (Sunderland, 1951).

TYPE II - Also called axonotmesis, refers to a loss of axonal continuity at the level of the lesion, although the endoneurial tubes are still intact. This type of lesion corresponds to a serious nerve compression that can interrupt the continuity of axons, resulting in Wallerian degeneration distally to the site of injury. It can also occur with the lingual nerve, mainly if the procedure of using the retractor is performed incorrectly, crushing the nerve against the lingual bone (Stacy, 1977). The time necessary for regeneration is usually short, determined by the distal growth of the axons through their intact endoneurial tubes towards the periphery. In these cases the prognosis for innervation of the correct targets is good because the endoneurial tubes provide direction for the growing axons (Gutmann et al., 1942-a).

TYPE III IV AND V - Also called neurotmesis, describes a condition in which some or all the connective tissue of the nerve trunk has been interrupted, including endoneurial tubes, perineurium and epineurium. In these cases, functional recovery involves the complex situation of axonal growth across a scar or a gap, since the essential collaboration of the endoneurial tube in guiding the growing axons is not present.

TYPE III- Consists of loss of axon and endoneurial tubes while the perineurium and the epineurium are still intact. Such an injury may result from severe compression or stretch injuries, causing axonal disintegration and Wallerian degeneration. The time needed for regeneration of type III injury is usually longer than for type II. This delay occurs at the site of the injury and is attributed to a lack of the endoneurial tube and larger intrafascicular disorganization (Gutmann et al., 1942-a).

TYPE IV - Refers to a loss of continuity of the endoneurium and perineurium with preserved epineurium. Injuries types III and IV are related to stretch injuries more common when a mucoperiosteal flap is raised in the mental region causing damage to the mental nerve (Robinson, 1992).

TYPE V- Indicates loss of continuity of the entire nerve trunk. It usually occurs when the nerve is transected. It can also occur with the lingual nerve if incision is made on the lingual aspect of the mandible and the nerve is running at the level of or higher than the alveolar crest (Kiesselbach and Chamberlain, 1984).

## ETIOLOGY OF LINGUAL NERVE DAMAGE

The most common cause of lingual nerve damage is injury during removal of the lower third molar (Mozsary and Middleton, 1984; Rood, 1983). The damage can vary from crush to total nerve transection usually caused by poor flap design, uncontrolled instrumentation, or fracture of the lingual plate (Blackburn and Brammley, 1989). Other causes like fibrosis, chronic irritation and repeated inflammation on the floor of the mouth due to denture hyperplasia have been reported (Mozsary and Middleton, 1984). Perforations made in the lingual plate in order to attach screws for rigid mandibular fixation following ramus osteotomy have also been documented as a cause of lingual nerve injury (Hegtvedt and Zuniga, 1990).

Schwartz (1973) provided a detailed classification of the causes of lingual nerve damage. He conducted a survey of twenty-five hundred members of American Society of Oral Surgeons. Of 1291 responses received, 418 cases of permanent and 2399 cases of temporary anaesthesia were reported. Of those responding, 860 (66%) oral surgeons reported cases of lingual anaesthesia following third molars extraction. The suggested causes were classified into 18 groups as is shown in table 1. The most common causes were the local anaesthesia needle passing through the nerve (28.72%), anatomical anomaly of the lingual nerve (11.96%) and retraction of the lingual flap (11.49%).

| | |
|---|---|
| Needle injury | 28.72 |
| Anatomical variation of the lingual nerve | 11.96 |
| Retraction of the lingual flap | 11.49 |
| Absence of the lingual plate | 10.40 |
| Fracture of the lingual plate | 7.92 |
| Dental follicle adhering to lingual tissue | 5.59 |
| Lingual flap design | 4.35 |
| Tongue retraction stretching the lingual nerve | 3.57 |
| Deeply placed sutures into the lingual flap | 2.95 |
| Lingual exploration to retrieve displaced root | 2.64 |
| Bur injury removing distolingual bone | 2.64 |
| Extension of root through the lingual plate | 1.86 |
| Bur passing through the lingual plate | 1.71 |
| Postoperative edema | 1.71 |
| Lingual nerve pinched to obtain haemostasis | 1.08 |
| Contamination by alcohol of anaesthetic solution | 0.47 |
| Formation of scar tissue after extraction | 0.47 |
| Coincidental neural lesion | 0.47 |

**Table 1**: Causes of lingual nerve injury found by Schwartz (1973). The values are percentages of the classified causes.

Other reported causes are alveoloplasties or dental procedures on patients who have bleeding disorders. Minor injuries can cause extensive haemorrhage and scar tissue may form causing permanent compression and loss of sensory function. Malignant tumours in the floor of the mouth may require sacrifice of the nerve tissue (Mozsary and Middleton, 1984).

Mason (1988) found no single factor to be causative of lingual nerve damage. After 1040 lower third molar extractions, no significant difference was found related to; the age or gender of the patient, side of operation, operator or operator seniority, assistant or assistant seniority, the type of lingual retractor used whether hand-held or self-retaining, the instrument of bone removal whether bur or chisel, the method of surgical division of the tooth, and finally, the number of sides operated in each patient. However, the lingual splitting technique, in which bone in the lingual side of the mandible is removed, is associated with a higher incidence of lingual nerve injuries than techniques in which the lingual plate is retained. Depth of impaction, removal of distal bone and degree of inclination of the impacted teeth were associated with an increased incidence of lingual nerve injury (Mason, 1988).

Although there is some speculation regarding the factors that could be responsible for lingual nerve damage (Schwartz, 1973; Mason, 1988), it is difficult to determine the extent in which each factor contributes for lingual nerve injury following lower third molar extractions. If bone needs to be removed from the lingual aspect of the mandible, a retractor should be placed between the lingual bone and the overlying mucoperiosteum to protect the lingual nerve from injury (Stacy, 1977).

## INCIDENCE OF LINGUAL NERVE DAMAGE

Cogswell (1942) reported that, in up to 90% of cases, the removal of impacted wisdom teeth was associated with some kind of nerve damage. Recent authorities would suggest that these figures are exaggerated, though some surveys give a surprisingly high incidence (e.g., Mason, 1988, Blackburn and Bramley, 1989).

Rood (1983) reported an incidence of 6.6% of lingual sensory disturbance following fourteen hundred procedures using the lingual split technique. This approach requires the retraction of the lingual mucoperiosteal flap which increases the incidence of lingual nerve damage (Mason, 1988; Blackburn and Bramley, 1989). In Rood's study all the damaged lingual nerves eventually recovered.

Using the same technique, Rud (1970) found that a 1% incidence of lingual nerve injury was reported out of 718 lower third molar surgeries. Six out of the seven cases recovered after a few months, but one had persistent paraesthesia for two years and six months. No lingual nerve injuries were reported after 162 lower third molars extraction using the buccal approach. Rud (1984) tried to reassess the lingual split technique by carrying out a survey consisting of lower third molar extractions. An incidence of 2% was found when the lingual plate was removed, compared with an incidence of 3% when the plate was split but retained.

On the other hand, Von Arx et al. (1989) reported a rate of 22% paraesthesia of the lingual nerve when using the lingual-split technique in 550 patients. Bruce et al. (1980) found 1.1% lingual paraesthesia using the buccal approach in 990 patients of a wide age range. The lingual plate fractured in 2.0% of the cases and a significant correlation was found between age and occurrence of lingual paraesthesias.

Davis et al. (1983) modified the lingual splitting technique to minimize trauma to the lingual soft tissues during removal of impacted lower third molars. In the modified distolingual splitting technique the soft tissue is not separated from the lingual bone and the fragmented bone remains attached to the periosteum. Using this modified procedure (Hochwald et al., 11983), of 598 extractions of impacted mandibular third molars, 26 cases (4.3 %) resulted in lingual paraesthesia. All the lingual nerves recovered completely although one case took five months to resolve.

Blackburn and Bramley (1989) reported an incidence of 11% lingual nerve damage following 1117 cases of lower third molar removals by a variety of surgeons. Only procedures that involved the raising of the lingual mucoperiosteal flap, with or without bone removal, were included in the survey. The trauma commonly resulted in changes in lingual sensation, but this persisted and became permanent dysesthesia in only six people (0.5%). Recovery occurred after variable time period. Over half the patients showed full recovery within six weeks and the longest time to full recovery was 36 weeks.

Harn and Durham (1990) presented an incidence of .54 %, 51 cases out of 9,587, that produced complications resulting from conventional mandibular block injections given for restorative procedures. Six per cent of these complications lasted from six months to five years, the duration of these alterations would suggest serious nerve injury such as axonotmesis or neurotmesis.

## CLINICAL CONSEQUENCES OF LINGUAL NERVE INJURY

Damage to the lingual nerve can be very distressing to the patient and embarrassing to the dentist. Injury to the secretomotor and vasomotor fibres present in the combined nerve trunk does not seem to cause any symptoms. Damage to thermosensitive and mechanosensitive fibres, present in the lingual and chorda tympani nerves, results in ipsilateral sensory alteration from the anterior two-thirds of the dorsal and ventral surfaces of the tongue, from the mucous membrane, and gingivae on the lingual aspect of the mandible (Rezai and Bayley, 1988; Robinson, 1992). Because of the lack of sensation from the anterior two-thirds of the involved side of the tongue, the patient may bite or chew the tongue and problems with speech and feeding will eventually arise (Rezai and Bayley, 1988).

Damage to the taste fibres causes loss of taste from taste buds on the same side affected by the injury (Zalewski, 1981; Cowan, 1990; Robinson and Winkles, 1991). This symptom is not commonly perceived by the patient. Taste bud and fungiform papilla survival are trophically dependent on nerves (Zalewski, 1981). After damage to gustatory nerve fibres, degeneration and disappearance of these structures occur followed by redifferentiation of the structures upon reestablishment of reinnervation (Robinson, 1989).

The alteration of sensibility of the tongue can be classified as follows:

1- Paraesthesia - Generally described as altered sensation ranging from pain, cold, warmth, and numbness to tingling, prickling, and burning. Usually they are features of progressive, incomplete, or recovering nerve lesions. They are aggravated by cold, pressure or tension on the nerve trunk, particularly at the site of injury.

2- Hyperaesthesia - Also an altered sensation, but specifically heightened sensitivity. Normal stimuli generate a more intense sensation than normal. This may be due to disturbances of conduction in damaged or immature regenerating nerve fibres. It is common during recovery, but stressful to the patient.

3- Dysesthesia or hypoesthesia - An impairment of sensory perception, a condition in which sensation is lowered but not totally lost. This occurs in partial nerve lesions and during sensory recovery following nerve injury.

4- Anaesthesia - Total sensory loss, is an occasional and grave postoperative complication of lingual nerve damage.

## FACTORS AFFECTING THE NERVE RECOVERY

The extent of functional recovery after nerve repair varies considerably between individuals. The regenerative process is influenced by several local and systemic factors, most of which are not well understood. Even if successful growth of axons to the periphery occurs, regeneration into inappropriate endoneurial tubes may result in false interpretations of the stimuli by the sensory cortex (Lundborg, 1988; Medinaceli and Seaber, 1989).

The recovery after crush is usually more complete than that after transection (Robinson, 1992-a). An experiment examining the rat dorsal ganglion (Peyronnard, 1988) shows a cell body loss of 45% after transection but no loss after crush. After crush the endoneurial tubes remain aligned and direct the growth of axons toward their appropriate target. In addition, the delay in the outgrowth is shorter and axonal growth proceeds faster (Medinaceli and Seaber, 1989). After nerve transection the "intrinsic guide lines" are interrupted, and whether the axon sprouts reach the correct receptor is not known (Sunderland, 1978). Moreover, transection leads to a rapid change in the endoneurial environment. Freed et al. (1985) transected and repaired the sciatic nerve of rats with the purpose of optimizing local environmental factors. The results are significantly better than the results achieved by routine microsurgical techniques. However, functional recovery never approached the original levels and none of the cases the animals recovered as rapidly as they did after crush injury.

# MORPHOLOGICAL CONSEQUENCES OF PERIPHERAL NERVE DAMAGE

## INTRODUCTION

Following peripheral nerve injury, simultaneous changes occur in the fibres proximal and distal to the site of damage, and in the neuron cell body (Sunderland, 1978; Lundborg, 1988).

When an axon is transected the distal segment is separated entirely from its supporting cell body and degenerates, so called Wallerian degeneration (Lubinska, 1982). Axons and myelin disintegrate, but the Schwann cells and their basal laminae persist. The first stages of this process are characterized by the disintegration of axons, axoplasmatic microtubules, microfilaments, and myelin sheath (Lubinska, 1982). Myelin degradation begins in the autophagic vacuoles of Schwann cells. Later macrophages take part in removing debris. The Schwann cells from the distal segment then multiply within their basal lamina to form longitudinal columns called bands of Büngner (Holmes and Young, 1942). The bands of Büngner play an important role in guiding the regenerating axons and providing a trophic source (Weinberg and Spencer, 1978). As the regenerating axons grow from the proximal stumps and elongate through these columns, they are ensheathed by Schwann cells and their calibre gradually increases. However, if there is a large gap between the two stumps of a transected nerve, the longitudinal extension of axons is threatened, recovery is impaired and a neuroma may form. A neuroma is a tangled mass of axons and supporting cells. Macroscopically, it is a swelling that develops in some circumstances at the end of the proximal stump of a transected nerve. It is a part of an injured nerve in which the Schwann cells and the endoneurium has been broken and regenerating axons have escaped to grow into the connective tissue of the nerve trunk (Gutmann and Medawar, 1942-a).

## AXON POPULATIONS AROUND INJURY SITES

One way of studying the recovery of nerve fibres from injuries is to look at axonal populations on each side of the injury site. Well-organized fibres distal to an injury site would suggest regeneration. Loss of fibres proximal to the injury site would show neuronal loss.

This technique has the disadvantage of not being able to detect axonal regeneration down inappropriate endoneurial tubes. Moreover, it is not possible to be certain about the fate of the cell bodies.

A degenerative reaction also occurs proximally to the site of damage. It follows the same pattern as that occurring distal to the site of damage and it extends centrally for a distance that varies according the severity of the injury. In injuries that do not rupture the endoneurial tube, retrograde degeneration usually restricts to the site of injury. In more severe injuries the effects might extend centrally for several centimetres (Sunderland, 1978).

The fate of a nerve fibre proximal to the site of damage depends upon the reaction of its corresponding cell body. It may survive the trauma and regenerate or it may degenerate. When cell death occurs, the proximal section of the nerve fibre undergoes complete Wallerian degeneration. If the cell body survives and regeneration occurs, the response of the cell body usually ends when appropriate peripheral connections are made (Sunderland, 1978).

## MYELINATED AXONAL POPULATION CENTRAL TO DAMAGE

Nearly all studies conclude that there is no change in the number of myelinated axons central to the damage site in the peripheral nerves of adult cats and rats. This is true for both crush injuries (Peyronnard et al., 1988) and transection injuries (Horch and Lisney, 1981; Carter and Lisney, 1987; Lisney, 1988; Holland and Robinson, 1990-a). It is also the case after transecting the nerve and allowing a neuroma to form (Peyronnard et al., 1986, 1988), and after removing a 10-mm segment of the nerve (Pover and Lisney, 1988).

The inferior alveolar nerve regenerates well following removal of a 10mm segment of the nerve in the mandibular canal of adult cats (Fried and Erdélyi, 1982). One week following the initial surgery, occasional unmyelinated axons showed signs of degeneration. There was no evidence of myelinated axonal degeneration. Pover and Lisney (1988) found no significant change in the number of myelinated fibres proximal to the injury of the sural nerve in adult cats. No alteration concerning the number of myelinated fibres was detected after transection, transection and repair by epineurial sutures, transection and repair by stump sutures, after removing a 10 mm nerve segment, and after removing a 10 mm nerve segment and repairing it with an autograft.

However, Risling et al. (1980) reported a loss of 15-30% myelinated axons in the ventral and dorsal root in kittens following transection of the sciatic nerve. There were signs of myelinated axon degeneration in the ventral roots at twelve and forty-nine days after damage. Risling and his colleagues (1983-a) carried out further experiments to investigate whether these findings were age dependent. They concluded that their previous findings were related to the developmental stage of the animals. Apparently nerve fibres degenerate more readily in response to damage in young animals than in adult animals.

After sciatic transection in kittens there is a reduction in the number of myelinated and unmyelinated axons (Risling et al., 1980). However, the reduction of unmyelinated axons is much greater after sciatic transection and previous neuroma excision (Aldskogius and Risling, 1983). This suggests that at least, some of these recurrent axons turn around in the neuroma. This reduction still outnumbers the number of cell bodies in the dorsal root ganglia showing that some sprouts originate at more proximal levels. Moreover, the fact that sciatic transection followed by neuroma excision in the kitten leads to a further reduction in the number of unmyelinated axons proximal to the transection (Aldskogius and Risling, 1983) when compared to sciatic transection alone (Risling et al., 1980), suggests that the unmyelinated fibres are more likely to grow back towards the central nervous system as recurrent axons.

## NUMBER OF UNMYELINATED FIBRES PROXIMAL TO DAMAGE

Although a great deal of information is available concerning the response of myelinated axons to injury, little has been said about damage to unmyelinated fibres (Dubner, 1978). The results published are controversial. The lack of agreement between studies are related to the difficulty in quantifying unmyelinated axons. After crushing lesions of the sural nerve in rats, there was no loss of unmyelinated axons proximal to the injury (Peyronnard et al., 1988). Carter and Lisney (1987) showed a 40% reduction in the number of unmyelinated axons proximal to the transection site. This experiment was done in the rat saphenous nerve after a recovery time of 26 weeks. On the other hand, others (Peyronnard et al., 1988; Holland and Robinson, 1990-a) did not report any decrease. Transection of the inferior alveolar nerve was followed by an initial increase in the number of unmyelinated axons. Eleven months after injury the values returned close to normal levels (Fried and Erdélyi, 1982).

When Peyronnard et al. (1986) transected and ligated the rat sural nerves at the ankle, the number of unmyelinated fibres proximal to the injury remained normal between two and fifty-two weeks later. A slight decrease was reported at later survival times (66 and 80 weeks).

The divergence between the findings of Carter and Lisney (1987) and the others could be explained by the fact that specimens were taken from different sites along the nerve relative to the site of injury (Lisney, 1989). For instance, in Peyronnard's experiments sections were taken 30mm central to the transection, while Carter and Lisney (1987) took samples only a few millimetres proximally. If the neurons do not degenerate completely, but instead, die back a few millimetres the data from Peyronnard and Lisney would be compatible. However, others (Holland and Robinson, 1990-a) took nerve samples several millimetres from the site of injury and did not find any drop in the number of unmyelinated axons.

To clarify the possibility of dying back of the unmyelinated axons, Lisney (1989) transected the sural nerve at the ankle level in rats. Counts of unmyelinated axons from sections taken a few millimetres proximally from the injury site showed an average loss of 24%. Whereas an average loss of 18% was reported when the site analyzed was 30 millimetres proximally to the injury site. These results indicate that a few axons died back but survived, and suggest that 18% of neurons died. Another possible explanation is that in some experiments (Carter and Lisney, 1987) nothing was done to facilitate regeneration, while in others (Holland and Robinson, 1990-a; Peyronnard, 1986) the nerves either remained in apposition or were sutured. This is puzzling because there was no unmyelinated axonal loss from nerves that had been transected and prevented from regenerating (Peyronnard et al., 1988).

After sciatic transection in adult cats, there was no unmyelinated axonal loss between thirty-five and one hundred and ninety days after injury (Risling et al., 1983). However, there was a reduction in the number of cell bodies in the dorsal root ganglia. This suggests that the reason no axonal loss could be detected is that recurrent or collateral sprouting from the proximal nerve stump occurred. There was an increase in the number of unmyelinated axons, between two and four months, after removing a 10-mm inferior alveolar nerve segment (Fried and Erdélyi, 1982). Other study (Aldkogius and Risling, 1983) showed that recurrent sprouts may invade the dorsal and ventral roots masking any loss of unmyelinated axons.

## CELL BODY COUNTS

There is a general agreement that some peripheral sensory neurons die after nerve injury (Risling et al., 1983;

Aldskogius and Risling, 1983; Peyronnard et al., 1986; Ygge, 1989). Ygge (1989) investigated the number of neurons lost from dorsal root ganglia comparing the effects of sciatic nerve transection at two different sites. There was an average neuron loss of 27%, when the transection was more proximal, versus 7% when the transection was more distal.

An attempt to find changes in the trigeminal ganglia, found no significant difference in cell body counts between unoperated and transected inferior alveolar nerve in cats whose nerves were allowed to regenerate (Holland and Robinson, 1990-b). When regeneration was prevented, there was no difference in the number of cell bodies between operated and unoperated side. There was a significant increase on the unoperated side. The reason for that surprising increase probably lies in the counting method (Holland et al., 1990-b).

Another study demonstrated that sectioning the inferior alveolar nerve and preventing it from regenerating, did not alter the cell size distribution of ganglionic cells (Ley, Holland and Robinson, unpublished). This suggests that transection does not damage any one size of cell bodies selectively.

## NUMBER OF MYELINATED AXONS DISTAL TO DAMAGE

The number of myelinated axons found distal to the injury estimates the number of axons that have successfully regenerated across the injury. Survival axons in the proximal stump, which fail to grow through the Schwann cell columns, become part of the neuroma that forms at the injury site (Sunderland, 1978).

Horch and Lisney (1981) investigated the recovery of the myelinated axons of sural nerve, using electrophysiological and morphological techniques. There was no myelinated axon loss proximal to the lesion after crushing, transecting, or removing 1-2 mm of the nerve. Counts of myelinated axons distal to the injury were 50% higher than proximal counts for the transected group. This shows that some axons in the proximal stump support more than one axon distally. This also occurred when 1-2 mm of the nerve was removed, though the increased counts were somewhat lower (20%). The picture was a little different when electrophysiological techniques were employed. Although crush injuries revealed that all the myelinated fibres that were morphologically present distal to the injury could be electrically excited eighteen months after the injury. Nerve transection showed that approximately 87% of the fibres that regenerated across the injury could respond to stimulation. The average dropped to 75% after transecting and removing a 1-2 mm nerve segment.

Other studies (Jenq and Coggeshall, 1984; Pover and Lisney, 1988) also revealed an increase in the number of myelinated axons distal to the injury. After removing a 10-mm nerve segment of sural nerve, the myelinated axon counts distal to the site of injury were elevated (Pover and Lisney, 1988). However, electrophysiological studies demonstrated that only about 40% could be stimulated from the skin after one year (Pover and Lisney, 1988). Distally, myelinated counts increased after a variety of lesions including: 1) cut with no attempt of repair; 2) cut and repair by epineurial suturing; 3) cut and repair by stump suturing; 4) removal of a 10-mm segment; 5) removal of a 10-mm segment and repair by stump mobilization and epineurial suture; and finally, 6) removal of a 10-mm segment and repair with an autograft. This increase was more prominent in the three first groups than in the three last ones (Pover and Lisney, 1988). The proportion of these fibres that could be excited by electrical stimulation of the nerve distal to the site of injury, was significantly lower in all of the groups. Group 1, the transected group and the repaired groups (groups 2 and 3) had better recovery than three last groups. A larger proportion of the fibres responded to stimulation in the first 3 groups than in the last three groups. They are 57%, 67% and 71% respectively. The repaired groups (groups 2 and 3) had better recovery than group 1 that did not have any repair.

Following successful regeneration, the number of myelinated fibres distal to the injuries is increased due to the axonal branching (Lisney, 1987; Pover and Lisney, 1988).

## NUMBER OF UNMYELINATED AXONS DISTAL TO DAMAGE

Following transection injury without repair 80% of the unmyelinated fibres that survive the injury in the proximal stump cross the injury within three months (Lisney, 1987). There is a considerable loss of unmyelinated axons from the proximal stumps of damaged nerves (about 40%) (Carter and Lisney, 1987). This suggests that only about half the original unmyelinated fibre population will be present in the distal stump after transection.

Fried and Erdélyi (1982) found a slight increase in the number of unmyelinated fibres distal to the injury 4 months after intra mandibular neurectomy. These values had declined to below normal levels 7 months after transection. They suggested that the increase detected four months after injury was due to branching of the myelinated fibres.

Unmyelinated axonal counts in a tributary of the sciatic nerve were significantly reduced after cut, crush and removal or 4-mm or 8-mm segment. The loss was greater with more severe injuries. In the other nerve tributary there was an increase of the number of fibres. This increase was only significant following cut and crush injuries (Jenq and Coggeshall, 1984).

## AXON SIZE AND MYELIN THICKNESS PROXIMAL AND DISTAL TO DAMAGE

Fibres in the proximal stump of a transected nerve undergo a reduction in diameter (Gutmann and Sanders, 1943; Weiss et al., 1945; Cragg and Thomas, 1961; Aitken and Thomas, 1962), which increases if the appropriate connections with the periphery are not made.

The change in the size of nerve fibres proximal to the site of injury has been attributed to either the outflow of axoplasm that occurs during regeneration, or to the failure of regenerating axons to establish functional connections with the periphery (Gutmann and Sanders, 1943; Weiss et al, 1945; Aitken and Thomas, 1962). This last suggestion supports the possibility that neurons can survive in an atrophic but stable state being responsible for some of the atrophied fibres found proximally to the site of damage.

The retrograde changes in fibre size following nerve injury correlates with the altered conduction velocities following injury (Cragg and Thomas, 1961). Many observers have noticed that axon diameters in the central stump become reduced during regeneration (Gutmann and Sanders, 1943; Weiss et al, 1945).

Several authors reported that myelinated fibres in the central stump following transection and suture have reduced diameters. Even in the late stages of recovery up to 300 days after injury (Gutmann and Sanders, 1943; Aitken and Thomas, 1962; Gutmann and Holubar, 1951). Following crush injuries the reduction in diameter was present for 130 days, but the fibre size returned to normal between 200 and 300 days (Gutmann and Sanders, 1943).

Alterations in axon diameter, thickness of the myelin sheath and nerve conduction velocities were studied by Cragg and Thomas (1961). They analyzed sites proximal to crush, constriction, cut and suture, or cut and avulsion of the distal stump. For survival periods of 7-50 days there was an average reduction in total fibre diameter of 5.3%. The myelin sheath became thicker by 5.9%. Between 50-150 days there was a mean reduction of 10.2% of the total fibre diameter and the myelin sheath increased by 3.5% in all four types of injuries. For survival times exceeding 150 days for the three first types of lesion the total fibre diameter increased such that after 225 days the axonal diameters were close to normal. A neuroma was observed in the fourth type of injury. There was a mean reduction of 15% in the total fibre diameter although the thickness of the myelin sheath was close to normal. Aitken and Thomas (1962) also found a significant decrease in the axonal fibre diameter. In their experiments there was a significant decrease in the myelin sheath at later stages.

In Cragg and Thomas' work (1961) sections were taken 8cm proximally to the lesion. This indicates that changes in diameter and myelin thickness were present even when the examination of the proximal stump extended up to 8 cm proximal to the lesion. These changes are not confined to the immediate area of the damage. They were also able to demonstrate that conduction velocity is related to axonal diameter. Other studies show divergent results. Reduction in myelinated fibre size are still present 12 months after unrepaired transection (Gutmann and Holubar, 1951) and after transection with suturing (Aitken and Thomas, 1962). The reduction in the myelin sheath was considerably greater than the reduction of the axonal diameter (Gutmann and Holubar, 1951).

A more recent report (Horch and Lisney, 1981) demonstrated that after nerve crush the conduction velocity proximal to the injury was reduced. Values were close to control levels six months after transection. Eighteen months after nerve section, the conduction velocities were close to normal. This was associated with a slight decrease in the number of A α, larger myelinated nerve fibres. Risling et al. (1983) showed a decrease in the proportion of A α fibres and an increase of medium-size fibres following sciatic nerve transection. They examined the ventral and dorsal roots after survival time of 35-190 days.

The axonal size distribution and the thickness of myelinated fibres distal to transection remain significantly reduced even at late stages of recovery (Fried and Erdélyi, 1982). Horch and Lisney (1981) demonstrated that, after transection and 18 months recovery time, there was an increase in the A γ group and a decrease in the A α group of fibres distal to the transection.

The conduction velocities distal to crushing injuries reach values close to normal (Horch and Lisney, 1981; Robinson, 1989). However, following nerve transection the conduction velocities were markedly reduced after 12 weeks (Robinson, 1989) and after 18 months (Horch and Lisney, 1981).

## SCHWANN CELL UNIT PROXIMAL AND DISTAL TO DAMAGE

Carter and Lisney (1981) reported a significant change in the distribution of Schwann cell unit sizes between proximal and distal sections. Sections were analyzed six months after transection of the saphenous nerve of the rat. They found a significant decrease in the size in Schwann cell unit size proximal and distal to the transection, but distally the changes were more dramatic. Holland and Robinson (1990-a) also observed a decrease in Schwann cell unit size proximal to inferior alveolar nerve transection in cats. Three months after the injury there was a decrease of the Schwann cell unit proximally to the injury, but not statistically significant.

On the other hand, another study reported an increase in number of umyelinated axons per Schwann cell after inferior alveolar nerve transection in cats (Fried and Erdélyi, 1982). Counts proximal to the injury after a 10-mm nerve segment removal were elevated between two and four months after injury. Eleven months after injury the counts proximal to the injury had decreased. However, control values for unoperated animals were not measured.

Multiple unmyelinated axons commonly occupy a single Schwann cell distally to the injury in regenerated nerves (Jenq and Coggeshall, 1985; Holland and Robinson, 1990-a).

# NERVE REPAIR

## INTRODUCTION

Several methods have been developed to improve nerve recovery (Medinaceli and Seaber, 1988; Melville et al., 1989). These methods vary from decompressions to a variety of forms of nerve grafting. The results range from satisfactory to poor (Sunderland, 1978).

Many factors influence nerve repair including; survival of the nerve cell body, initiation of sprouting, growth of sprouts across the zone of injury, reinnervation of endoneurial tubes in the distal segment, reinnervation of appropriate targets and the maturation of nerve fibres, (Gutmann et al. 1942-a, 1942-b). Some factors that may be responsible for poor results after nerve repair include: resection associated with severe wounding, coexisting arterial damage, infection, delay before repair and tension at the suture line. The extent to which each of these may contribute to poor repair is not clear (Sunderland, 1978).

The results after nerve repair are determined by the randomness of regrowth at the site of injury (Medinaceli and Seaber, 1989). Medinaceli and Seaber (1989) suggested that the accuracy of the path taken by each regenerating axon is responsible for the quality of regeneration. This factor is important following lingual nerve injury due to the presence of fibres from several different sources. After transection and repair, the large variety of nerve fibres present in the trunk may predispose the growing neurites to enter inappropriate endoneurial tubes.

Other factors such as poor quality of the repair, mechanical stress caused by the suture thread, variations in the degree of remyelination, and variations in the amount of growth factor in target tissues have been reported as important factors determining the outcome of nerve repair (Ramon and Cajal, 1928). Unfortunately, most of these factors cannot be affected surgically and are beyond the ability of surgeons. The extent of nerve recovery is controlled by many factors that act central to the injury, at the suture line, and distal to it (Sunderland, 1978).

## FACTORS ACTING PROXIMALLY, DISTALLY AND AT THE SUTURE LINE

The key structure in determining the outcome of regeneration in peripheral nerves is the axonal sprout that develops following injury (Holmes and Young, 1942; Gutmann and Sanders, 1943; Lisney, 1988). This growth depends on the survival of the injured neurons (Lisney, 1989). After peripheral nerve injury, some cell bodies remain unaffected, others may degenerate, the remainder may show a variable "chromatolytic" response that is reversible (Sunderland, 1978). Complete axonal degeneration causes a reduction in the number of neurons that would be available for regeneration (Risling et al., 1983). Following nerve repair, recovery may be incomplete (Mozsary and Middleton, 1984). However, it is difficult to determine the extent to which permanent central changes contribute to partial recovery (Sunderland, 1978). The intensity of the retrograde reaction, the severity of the injury and the time taken for recovery to occur are important factors that will interfere with the effectiveness of axonal growth towards the periphery (Gutmann et al., 1942-a; Holmes and Young, 1942; Robinson, 1989).

The distance along which retrograde axonal degeneration occurs depends on the severity of the nerve injury. After crushing injuries it is restricted to the immediate neighbourhood of the injury (Lubinska, 1961). The internode degenerates if the transection is made in its proximal part (Lubinska, 1961). This probably relates to the preservation of the nucleated part of the Schwann cell.

Neurons respond better when injury occurs farther away rather than close to their cell bodies (ygge, 1989). After sciatic injury retrograde degeneration was less severe in motor than in sensory neurons (Risling et al., 1983). This has been attributed to the severity of retrograde degeneration and to the involvement of more axons. The reason more axons are involved at more proximal sites is probably that few or none of the axons left the main trunk to innervate the targets.

Despite these central alterations, most of the repaired nerves do send sprouts that attempt to reach the periphery (Holmes and Young, 1942; Pover and Lisney, 1988). All axons do not start sprouting simultaneously. The later growing axons may reach the distal stump when most of the endoneurial tubes have been already occupied by sprouts, causing competition for available endoneurial tubes (Sunderland, 1978). Moreover, axonal sprouts may enter and grow inside functionally inappropriate endoneurial tubes leading to the incorrect innervation of target organs (Medinaceli and Seaber, 1989).

Factors related to the diameters of the endoneurial tubes and fasciculi have an important role in determining the success of nerve repair (Sunderland, 1978). Holmes and Young (1942) observed shrinkage of the distal endoneurial tubes and fasciculi in the first four months after denervation. The shrinkage of the fascicles will concentrate the endoneurial tubes in a smaller area. This results in fewer regenerating axons entering the tubes than would have been the case if they had not shrunk. This is due to a greater chance of the proliferating axons growing into a larger area of connective tissue (Sunderland, 1978). Distal stumps denervated for one year presented endoneurial tubes that had become so reduced that it would be very difficult for new fibres to enter (Holmes and Young, 1942). Factors related to the variation in the stage of degeneration of the distal stump may influence the extent of recovery.

Holmes and Young (1942) designed an experiment in which the source of axons in the proximal stump was always freshly transected nerve being sutured with two different kinds of distal segments; 1) recently transected distal nerve segments and 2) distal segments that were denervated for one year period. The group that had distal stumps denervated for one year before the repair had a significant decrease in the number of axons per endoneurial tube. Holmes and Young (1942) suggested that this reduction was due to the atrophy of the lumen of the endoneurial tubes. They also reported an increase of endoneurial collagen that probably contributed to the reported results. Other factors acting distally include alteration of the size fibre diameter, variation in the degree of myelination (Gutmann and Sanders, 1943), and presence of nerve growth factor (Otto et al., 1987).

Various adverse factors acting at the site of injury are responsible for poor results after nerve repair. These factors are: mechanical tension caused by the effort in maintaining the stumps together (Gelberman, 1991), poor quality of nerve repair and formation of scar tissue (Medinaceli and Seaber, 1989). If scar tissue forms around the nerve preventing it from regenerating, microsurgical excision is the preferred treatment for restoring normal sensation to the cut or damaged lingual nerve (Mozsary et al., 1982; Donoff and Guaralnick, 1982)

Preparation of the proximal stump for repair has the capacity of inducing further retrograde changes in the surviving cells (Sunderland, 1978). It seems that the technique of nerve repair is only one of several factors that determine the functional outcome of nerve regeneration. Some techniques of nerve repair considerably improve nerve recovery (Medinaceli and Seaber, 1989; Mozsary et Middleton, 1984).

## METHODS OF NERVE REPAIR

The purpose of nerve repair techniques is to regain continuity of the proximal nerve trunk and its distal elements. It aims to reduce scar formation and allow the proper entrance of sprouts into appropriate endoneurial tubes (Lundborg, 1978). The compromising factor of mismatching after nerve repair, which is the entry of axons into inappropriate endoneurial tubes, can seriously affect the outcome of regeneration. An accurate apposition of the nerve internal structures would diminish the wasteful growth of axons into foreign endoneurial tubes (Medinaceli and Seaber, 1989). Unfortunately, there is no nerve repair technique that fits this requirement perfectly. Perineurial nerve sutures offer a better interfascicular apposition decreasing the chances of mismatching. The utilization of perineurial as opposed to epineurial nerve sutures is more traumatic and does not always improve the outcome of regeneration (Lundborg, 1988). There is not an ideal method of repair that would be appropriate for every type of nerve damage. Each type of injury has to be analyzed separately. The suitability of a specific technique will depend on the internal anatomy of the injured nerve (Gelberman, 1991).

## NEUROLYSIS

Neurolysis consists of freeing a nerve from the constricting scar tissue. If a scar tissue envelops a damaged nerve in continuity, simple neurolysis might be enough to obtain satisfactory results (Gelberman, 1991). Gelberman (1991) reported 33-60% success when external neurolysis were performed in nerve in continuity. There are two types of neurolysis, external and internal. External neurolysis consists of freeing the nerve tissue from scar tissue around the whole trunk. Internal neurolysis is performed around the fasciculi when scar tissue involves the interfascicular tissue (Sunderland, 1978).

Advanced technology and improved microsurgical techniques have made it possible to perform neurolysis safely. It is, however, possible to sever arteries while relieving the nerve trunk. During internal neurolysis, there is also a risk of mistaking the interfascicular vessel communication for scar tissue. If this is the case further scarring would warrant problems (Gelberman, 1991).

## EPINEURAL REPAIR

Epineural repair is a long standing method of repair in which sutures are placed in the epineurium to appose the ends without creating tension (Mozsary et al., 1984). It has the advantage of simplicity and of not involving internal structures such as the perineurium (Lundborg, 1988; Gelberman, 1991).

Longitudinal blood vessels are useful in helping to ensure appropriate matching. However, trimming of the nerve stumps may disturb the fascicular pattern leading to mismatching. It can also increase the distance between the two stumps causing tension at the repair site. The surgeon must assess the length of the nerve that needs to be removed. If suturing would cause significant stress at the repair site, the possibility of nerve grafting should be considered (Gelberman, 1991). In some cases after damage the cut ends can retract so much that suturing would be not recommended due to the enormous amount of pressure that suture repair would cause.

Nerve suture has been performed for lingual and inferior alveolar nerves (Riediger et al., 1989). Primary nerve suture has been employed after complete section of the lingual nerve and has been shown to improve sensation by 77% (Riediger et al., 1989).

Following sural nerve transection, Pover and Lisney (1981) reported a slightly better recovery after repairing the nerve using epineural suture. On the other hand, Gibby et al. (1983) suggested no fundamental differences in the success of regeneration between unrepaired and sutured radial or sural nerves, although much smaller neuromas were detected after suturing than after no repair. He also showed that application of anti-inflammatory steroids at the site of the repair did not improve the results.

## GROUP FASCICULAR REPAIR

This method is a more accurate technique for nerves that have groups of fascicles from unrelated sources. It consists of identifying the correspondent groups and unifying them by placing the suture in the internal perineurium. This technique had the advantage of reducing mismatching because axons have a higher chance of remaining in their fascicles across the suture innervating appropriate targets (Gelberman, 1991). This type of repair is more prone to internal scaring.

## FASCICULAR REPAIR

Fascicular repair or perineurial suturing consists of suturing individual fascicle together by placing sutures in the perineurium. This technique provides a good alignment of internal structures, which would increase the probability of axonal regrowth into appropriate endoneurial tubes. However, as with group fascicular repair, the inner structures of peripheral nerves are manipulated, increasing the chances of further scarring (Gelberman, 1991). Pure sensory nerves repaired with epineural sutures provided similar results as those repaired with the more meticulous method of fascicular repair. Fascicular lingual-chorda repair provides satisfactory results in humans (Mozsary et Middleton, 1984).

## NERVE RECONNECTION

This nerve technique utilizes an antiretraction device. This device applies force at some distance away from the cut ends alleviating stress at the tip of the stumps. (Medinaceli and Seaber, 1989). The results after sciatic nerve transection in rats always showed satisfactory recovery following nerve reconnection. Regeneration was also satisfactory when nerve connection was used as a secondary repair if any previously used technique showed good recovery. Unfortunately, it was not able to improve an unsatisfactory result when used as a secondary method of nerve repair.

## NERVE GRAFTING

Nerve grafting is indicated when tension-free adaptation of the nerve ends is not possible. Suture under tension affects regeneration by decreasing circulation, causing fibrosis and formation of scar tissue. When there is a large gap between the stumps, nerve grafting is often a good alternative. It behaves like the distal stump in regular repair, except that the axons have to cross two damage sites.

There are several types of nerve grafts and their classification is related to the source of the graft.

Autografts - Refers to nerve grafts from the same individual.

Allografts - Refers to nerve grafts from individuals from the same species.

Xenografts - Refers to nerve grafts between different species.

Fresh autologous nerve grafting is the most common type of graft due to the immunologic reaction that the allografts or xenografts cause. Sutures and fibrin glue are both recommended to keep the graft linked to the nerve endings (Gelberman, 1991).

Nerve grafting improves lingual sensation by 60% (Riediger et al., 1989). Autologous nerve graft following removal of a 10-mm segment of the sural nerve was successful following simple transection and epineural suture repair (Pover and Lisney, 1988).

Nerve grafts, unfortunately, have the potential of providing unpredictable results and creating sensory problems at the donor site. Other sources of grafts such as autologous vein and frozen muscle tissue have been employed to bridge gaps guiding growing axons between the two endings of transected nerves (Myles, 1992). The results obtained by autologous vein grafts (Pover and Lisney, 1988), and muscle grafts (Myles et al., 1992) are satisfactory.

## TUBULATION

Tubulation is a method of nerve repair that consists of inserting the proximal and distal nerve stumps into a tube. It has been made experimentally using various types of tubes, such as arteries, silicone and other resorbable or non-resorbable tubes. Sutures may be placed at the extremities of the tubes (Gelberman, 1991).

Entubulation has great advantages because it avoids the loss of nerve tissue. Resorbable tubes have another advantage, avoiding a second surgery to remove the tube.

Successful methods of tubulation using a hypoantigenic collagen sheet caused better skin innervation. This was significantly superior to no repair or suture when 8mm of nerve tissue was lost (Gibby et al., 1983).

Another study in rats shows no neuron loss after removal of 8mm and 15mm sciatic nerve segments and subsequent entubulation. When the stumps were not entubulated a significant neuronal loss was observed (Melville et al., 1989).

Despite extensive study, the ideal universal method of nerve repair remains unknown. The purpose of this study was to evaluate a method of nerve repair specifically on the lingual nerve.

# CHAPTER THREE

# METHODOLOGY

## SUBJECTS AND EXPERIMENTAL INJURY

The animal preparation and the electrophysiological recordings were made by Dr. Keith Smith at the University of Sheffield. Fourteen cats aged 18 to 28 months and weighing 3 to 4.5 kg were used. The animals were anaesthetized with a mixture of alphaxalone and alphadolone acetate (Saffan, Glaxo Laboratories). The dose used consisted of intra-muscular injection of 18 mg/kg and maintenance of 2 mg/kg intravenous) and intubated. The left lingual nerves were exposed and access obtained via an incision placed over the angle of the mandible. After the elevation and retraction of masseter muscle, the ramus of the mandible was perforated using dental burs and the medial pterygoid muscle was divided to expose the lingual nerve. The left lingual nerves were sectioned with a pair of scissors 3 to 6 mm distally to the lingual-chorda junction. The right side of the animals was left intact acting as a control (Figure 1). In 8 animals (Groups I and II) no repair was attempted, the wound was closed in layers with gut sutures and penicillin was given (Amphipe, Gist-Brocades, U.K.; 1.0ml i.m.). A final experiment was carried out to determine the extent of recovery, 12 weeks later in 3 animals (Group I) and 24 weeks later in 5 animals (Group II). The animals were anaesthetized with sodium pentobarbitone

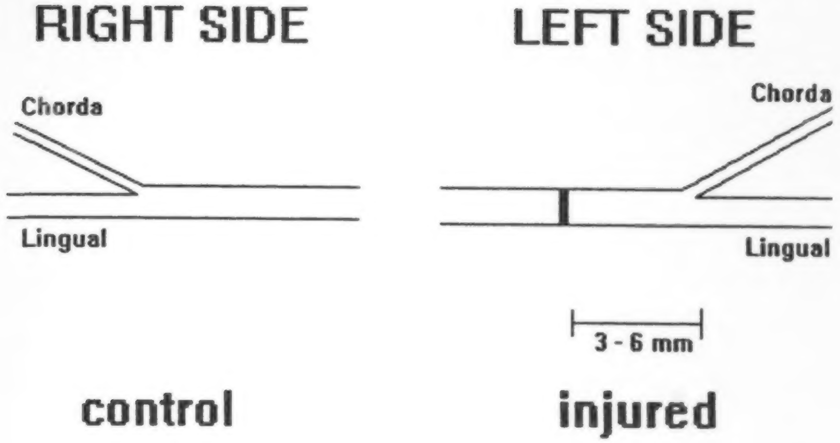

RIGHT SIDE
LEFT SIDE
Chorda
Lingual
Chorda
Lingual
3 - 6 mm
control
injured

**FIGURE 1:** Diagrammatic representation of the right and left lingual nerves illustrating the site of injury related to the lingual-chorda junction for groups I, II and III. The right lingual nerves were not operated. The left lingual nerves were transected with a pair of scissors 3 to 6 mm distal to the lingual-chorda junction.

anaesthesia, (induction of 42 mg/kg intraperitoneal and maintenance of 3 mg/kg intravenous), the trachea cannulated, and body temperature maintained with an electric blanket thermostatically controlled from a rectal thermistor. The right and left lingual nerves were again exposed and dissected. Electrophysiological recordings were made distally into the body of the tongue where the nerve was sectioned. The nerve segments were then placed in buffered glutaraldehyde for fixation.

A third group (group III) of 6 animals, underwent the same type of procedure as group I, except that after transection the nerves were immediately repaired with epineurial sutures [9/0 Ethilon-Monofilament polyamide (Ethicon Ltd. U.K.)], four or five sutures were necessary for keeping the stumps in apposition (Figure 2). Twelve weeks later a terminal experiment was carried out following the same parameters as for groups I and II.

As mentioned above, to expose the entire nerve segment, the lingual nerves were dissected to a more distal point into the body of the tongue. The dissected nerve segments were placed in a buffered glutaraldehyde vial and dispatched to Edmonton. Then, they were divided in smaller segments as is shown in figure 3 to allow the examination of sites proximal and distal to the injury.

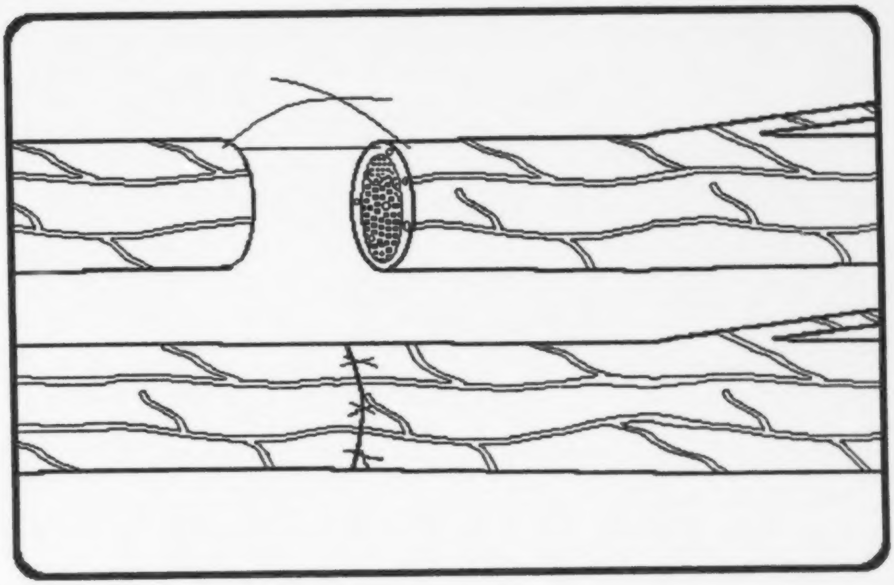


**FIGURE 2**: Diagrammatic representation of the repaired lingual nerves from group III (12 weeks, repaired). Four to five stitches were placed in the epineurium of the lingual nerves to avoid the separation of the two stumps.

To preserve their structural composition and provide analyses in the transmission electron microscope, the nerve segments were processed when they arrived from England as follows:

1- Washed 3 times, each time 5 minutes, in a solution of 0.2M of cacodylate buffer.

2- Fixed for 1 hour in a solution of 2% osmium tetroxide in 0.1M cacodylate buffer.

3- Washed 3 times, each time 5 minutes, in double distilled water.

4- Placed for 1 hour in a solution of saturated uranyl acetate in double distilled water.

5- placed for 1 hour in a solution of 2% uranyl acetate in 75% ethanol.

6- Dehydrated 2 times, each time 15 minutes, in 70% ethanol.

7- Dehydrated 2 times, each time 15 minutes, in 85% ethanol.

8- Dehydrated 2 times, each time 15 minutes, in 90% ethanol.

9- Dehydrated 2 times, each time 30 minutes, in 100% ethanol.

10- Placed 2 times, each time 15 minutes, in a solution of propylene oxide.

11- Placed in the rotator overnight in a solution of propylene oxide and araldite (50:50).

The nerve segments were then placed in fresh araldite and left overnight. The next day they were embedded in fresh araldite and placed in the oven at 37°C overnight. The following day the temperature of the oven was increased to

60°C, the specimens remained there for at least another day.

The segments A and C were then ready for sectioning. Semithin sections (1 μm thick) from faces 2 and 3 were taken for light microscopy and stained with toluidine blue. Ultrathin sections (60 nm thick) from faces 2 and 3 for electron microscopy were stained with uranyl acetate and lead citrate (Figure 3).

Sections were taken 3mm proximal and distal to the site of injury. In the control side, sections were taken 3mm distal to the lingual-chorda junction (Figure 4).

## MORPHOMETRICAL PROCEDURES

Although it is possible to count and measure all the axons present in the lingual-chorda trunk, it is extremely time consuming. Unbiased systematic sampling techniques allowing a faster examination were developed by Mayhew and Sharma (1984) and Mayhew (1988). Systematic sampling can be based on strips, axial strips, squares, or sectors (Figure 5). It consists of randomly selecting the first area, square or strip, to be examined. The subsequent areas are determined systematically by the position of the first selected area. Although a simple random sampling technique is also considered reliable (Mayhew, 1990), a systematic random scheme is preferable to avoid oversampling some areas (Figure 6). For the same reason, sectors are better than axial strips in not oversampling the middle (Mayhew and Sharma, 1984; Mayhew 1988)

(Figure 5).

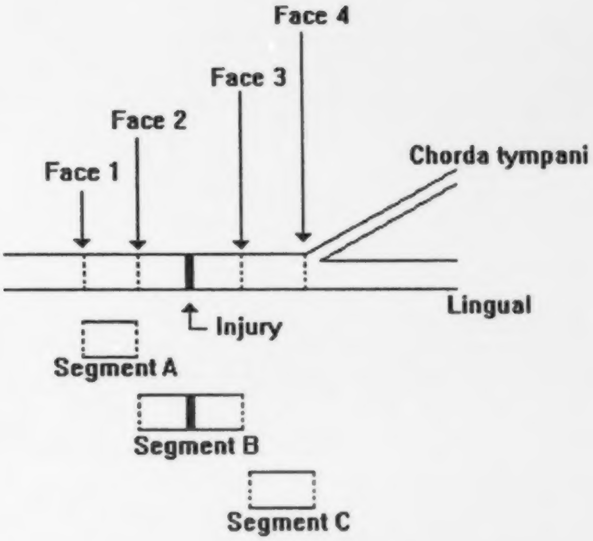

Face 4
Face 3
Face 2
Face 1
Chorda tympani
Lingual
Injury
Segment A
Segment B
Segment C

**FIGURE 3**: Diagrammatic representation of the lingual nerve segments that underwent tissue processing and embedding. Faces 2 and 3 from segments A and C were the analyzed sites proximal and distal to the injury in all the groups.

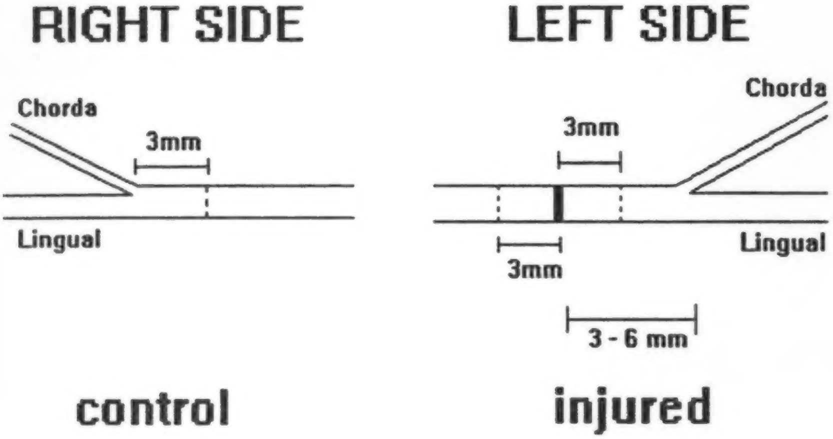

RIGHT SIDE
LEFT SIDE
Chorda
3mm
Lingual
Chorda
3mm
3mm
Lingual
3 - 6 mm
control
injured

**FIGURE 4**: Diagrammatic representation of the analyzed sites in the right and left lingual nerves. In the control side sections were taken 3mm distal to the lingual-chorda junction. In the operated side sections were taken 3mm proximal and distal to the injury.

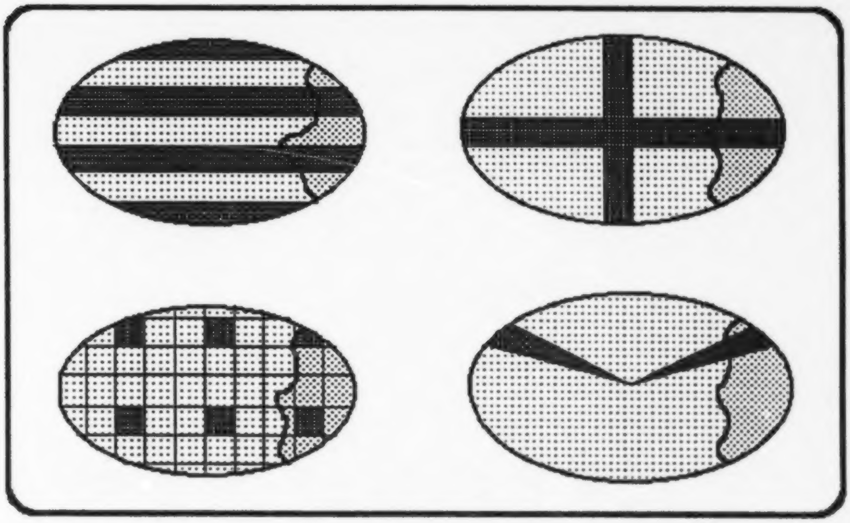


**FIGURE 5**: Diagrammatic representation of different systematic sampling techniques used by Mayhew and Sharma (1984). The figures represent a transverse section through a nerve trunk. The upper figures illustrate the systematic strip sampling (left) and the axial strip sampling (right) techniques. The lower figures represent the systematic random square sampling (left) and the systematic random sector sampling (right) techniques. Axons appearing in the dark areas were counted.

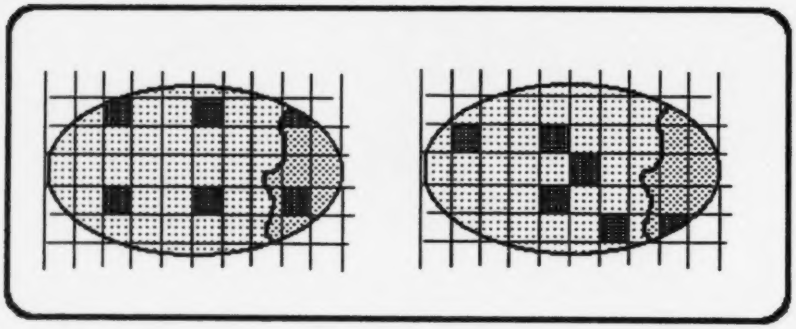


**FIGURE 6**: Diagrammatic representation of the systematic random sampling technique (left) and the simple random technique (right) tested by Mayhew and Sharma (1984). The figures represent a transverse section through the lingual nerve trunk. Axons located in the dark areas are counted.

Mayhew and Sharma (1984) and Mayhew (1988) tested all the above mentioned methods and the simple random square sampling technique in the tibial nerve of cats. They found all the systematic methods listed above as well as the simple random square sampling technique very reliable in preserving the characteristics of axonal size distribution. Although apparently the systematic square sampling had a more accurate result than the simple random square sampling technique, the differences between them were not statistically different.

## **VALIDATION OF THE METHODS**

To decide which of the above sampling techniques would be the best to estimate the axonal population of the lingual-chorda trunk, the myelinated size fibre population of the entire lingual nerve trunk was determined at the light microscopical level (Magnification X 2800). Then, the systematic square and systematic sector sampling techniques were employed in the same specimen using the electron microscope (Magnification X 8400). This magnification also allows adequate resolution for the estimation of the unmyelinated axon population.

To sample approximately 6% of the nerve trunk using the sector sampling technique, 2 randomly chosen strips passing through the centre of the trunk were photographed. These strips were wide enough to include an angle of 11.5 degrees. Then, the sectors were drawn from the centre of the trunk into the montages (Figure 7). Small axons have a greater chance of being totally inside a determined area than larger axons. Therefore, all the axons that lay completely or had their centres inside the selected area were considered. This method is unbiased, not favouring the small axons (Mayhew, 1990).

To sample 6 % of the nerve trunk using the systematic square sampling technique, the cross-sectional area of the nerve trunk was traced using a camera lucida and measured using a Based digitizer image analysis system, and appropriate software (Bioquant, R & M Biometrics, Nashville, Tenessee). To facilitate the systematic square sampling technique, a 300 mesh slim bar grid was employed. The random direction of the squares was determined by how the specimen happened to be placed in the mesh grid (Figure 8). Then, the number of squares to sample 6% of the nerve was calculated. The squares that contained the lingual and chorda-tympani axons were numbered and the first square was picked by random number selection. The other squares were determined sequentially from the first. For instance, if a specimen has 30 squares and five need to be photographed, every six squares from the first chosen will be picked. If the first chosen square was the square number 5, then the squares number 5, 11, 17, 23, 29 were selected.

There was no difference between the two sampling techniques chosen. They both revealed 5% more myelinated axons and a larger proportion (12%) of small axons (<3.5 micrometres

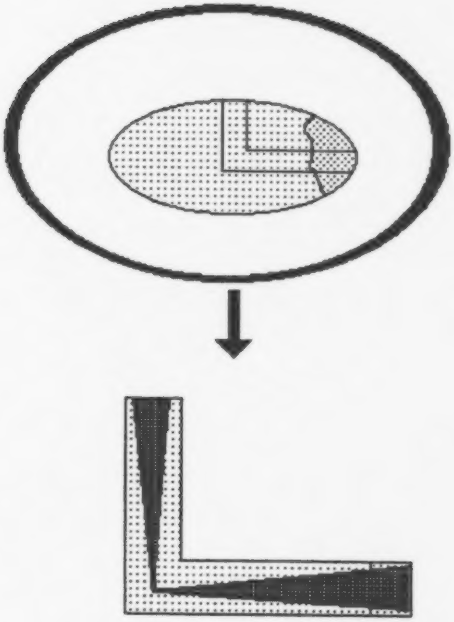


**FIGURE 7**: Diagrammatic representation of the systematic sector sampling technique tested. The upper figure represents a transverse section through the lingual nerve placed in a slot grid. The "L shaped" area was photographed. The lower figure represents the photographed area. Two angles of $11.5^0$ (dark areas) were drawn within these strips. The myelinated axons located within the sectors (dark area) were counted and measured.

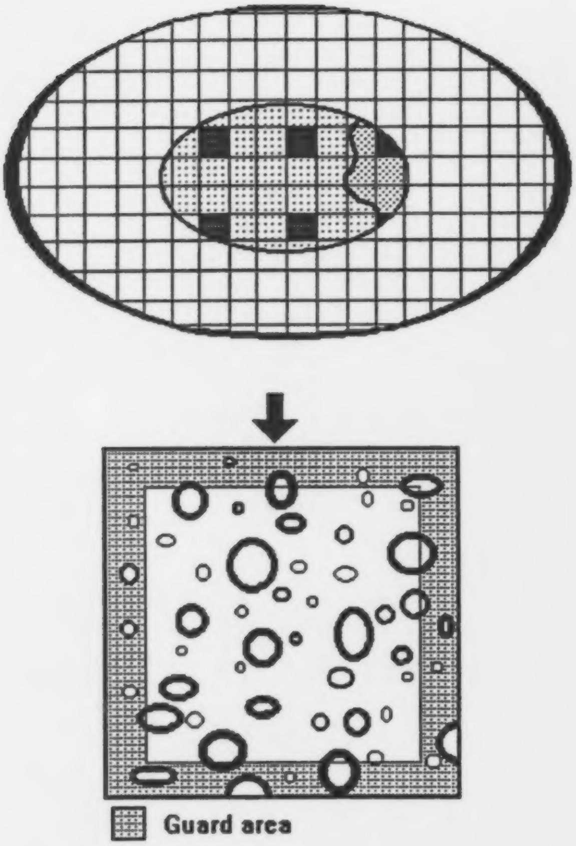


**FIGURE 8**: Diagrammatic representation of the systematic square sampling technique. The upper figure represents a transverse section through the lingual nerve trunk placed in a mesh grid. The smaller bundle located in the left is the chorda tympani nerve and the larger bundle is the lingual nerve. Axons that appeared in the dark squares of the lingual and chorda tympani nerves were counted. The lower picture represents a selected square and its guard area. All the axons that had their centres within the smaller square were counted and measured.

diameter) than the method that counted and measured all the single myelinated axons. The mean circumference of the myelinated axons was 22.91 (10.66) µm when all the axons were measured, 19.11 (8.95) µm for the square and 19.07 (8.54) µm for the sector systematic sampling techniques (Table 2). This might be due to a higher magnification (X 8400) being used in the sampling approach revealing small myelinated axons that may have been missed when all fibres of the nerve trunk were measured at light microscope level (x 2800).

| | **NUMBER** | **SIZE MEAN** | **S.D.** |
|---|---|---|---|
| **1- TOTAL** | 4093 | 22.91 | 10.66 |
| **2- SQUARE** | 4436 | 19.11 | 8.95 |
| **3- SECTOR** | 4313 | 19.07 | 8.54 |

**Table 2:** The number and size of myelinated axons present in the lingual nerve for the three different procedures tested. 1- All the myelinated axons were counted and measured at light microscopy (TOTAL). 2- Six per cent of the nerve trunk was sampled using the systematic square sampling technique at electron microscopy (SQUARE). 3- Six per cent of the nerve trunk was sampled using the systematic sector sampling technique at electron microscopy (SECTOR). Obs.: The values are: Number of myelinated axons, Mean size and Standard Deviation (S.D.) of the myelinated axons.

Mayhew and Sharma (1984) and Mayhew (1988) found no difference in the mean size of myelinated axons between sampling techniques tested and the conventional method. However, in his studies the same magnification was used for both procedures.

The method of systematic square sampling was adopted in preference to sector sampling because it is well recommended for nerves of mixed fascicularity (Mayhew and Sharma, 1984). As may be the case of nerve after injuries. It also facilitates the sampling of the chorda tympani when this is distinct from the lingual nerve.

## DATA COLLECTION

Semithin and ultrathin sections from sites 3mm proximal and 3mm distal to the injury were cut using an ultramicrotome (Sorval MT-6000 - Wilmington, Delaware 19898 U.S.A.) (Figure 4). Semithin sections were examined in the light microscope (Zeiss West Germany - Carl Zeiss D-7082 Oberkochen) and the perimeter of the nerve trunk was traced with a camera lucida. Using a Based image analysis system and appropriate software (Bioquant R & M Biometrics, Nashville, Tennessee), the total area of the nerve, excluding the epineurium was measured. Ultrathin sections were cut and mounted on carbon coated slot copper grids. Depending on the area of the nerve trunk a 300 or a 400 copper mesh Slim Bar Grid was placed on top of the slot grid. The number of squares needed to sample 6% of the nerve trunk was estimated and the sections were examined in a Hitachi 7000 electron microscope. Photographs were assembled and a smaller square was drawn on each montage establishing the guard area (outside contour around the smaller square)(Figure 8). The guard area allowed the measurement of all the axons, whose centre lay inside the smaller square, which was the area sampled. After measuring the diameter and the thickness of all the myelinated axons present in the selected squares, the number of unmyelinated axons per fibre was counted. The total number of axons in the entire nerve trunk was calculated from the number of axons present in the 6% of the nerve sampled.

## ANALYSIS OF THE DATA

The following data were collected: The number of myelinated axons, their diameter and the thickness of the myelin sheath from control, proximal and distal sites. The total number of unmyelinated axons and their number present within each Schwann cell (Remak Bundles) were also recorded from control, proximal and distal sites. Data from sites distal and proximal to the injury were compared statistically with each other and to the control side of each animal.

The size distribution of myelinated axons and the thickness of its myelin sheath is positively skewed (Holland and Robinson, 1992). Therefore, a non-parametric paired test (Wilcoxon test) was used to detect any significant difference of the measured parameters between control and proximal, control and distal, or proximal and distal sites. The use of the Wilcoxon test for discrete data (number of axons) was possible due to the large number of axons ($n>10$) present in the nerve trunk (Ott, 1977). The data was described in terms of means, standard deviation and standard error of the means. A p-value $< 0.05$ was considered significant.

# CHAPTER FOUR

# RESULTS

## INTRODUCTION

In all cases the injured nerves recovered. A neuroma "in situ" wider than the proximal and distal nerves was present at each injury site. In unoperated nerves the lingual and the chorda tympani are readily distinguished. Proximal to the injury, it was possible to distinguish between lingual and chorda tympani nerves in groups II and III (Figure 9). However, in group number I in only one specimen was the distinction clear (Cat number 2 from group I). There were no other apparent qualitative differences between proximal and control sections. Distal to the injury, it was not possible to distinguish between the lingual and chorda tympani nerves for all animals from groups I and III (Figure 10) and all except one animal from group II. Group II included one animal in which the lingual and the chorda tympani nerves were clearly separated (Cat number 1 from group II) (Figure 11). The nerves distal to the injury were polyfascicular in most cases. Group I had a case in which the nerve was bifascicular. Group III had another case in which the nerve was monofascicular. On the other hand, group II had only one case in which the nerve was polyfascicular.

## NUMBER OF MYELINATED AXONS

The total number of myelinated axons in the following tables refers to the number of myelinated axons found in all the component nerve samples analyzed with no distinction being made between the lingual and the chorda tympani nerves. Comparisons between control and proximal sections in groups II and III were made for the lingual, chorda tympani and total values. In group I comparisons between control and proximal sections were only possible with total values. In all groups comparisons between control and distal, as well as proximal and distal were possible only with the total values.

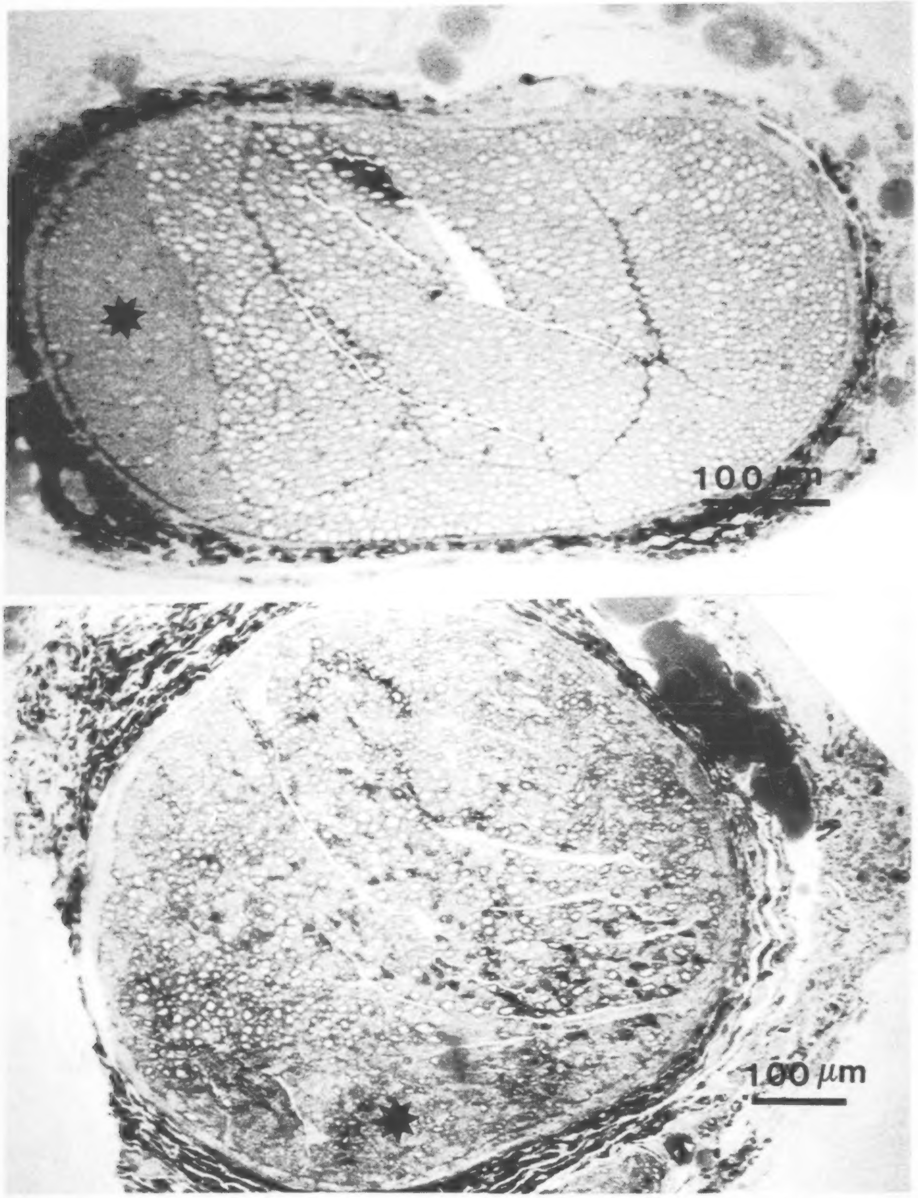


**FIGURE 9**: Transverse sections of the combined lingual and chorda tympani nerves (light micrographs, toluidine blue). The upper micrograph is from the control side, the lower is from proximal to the transection of group II (24 weeks, unrepaired). It is possible to identify the lingual and the chorda tympani nerves in both. The chorda tympani(✱) in the control section (upper) is located in the left side and in the proximal section (lower) at the bottom of the picture.

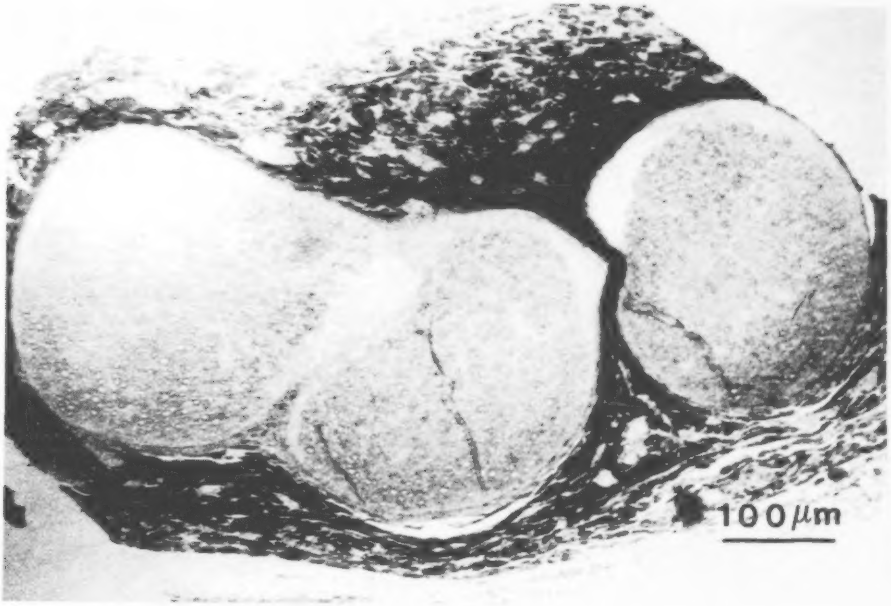


**FIGURE 10**: Transverse section of the lingual nerve distal to the transection from group III (12 weeks, repaired)(light micrograph, toluidine blue). It is not possible to identify the lingual and the chorda tympani as independent nerve trunks. All the three fascicles had the same size of axons being impossible to make a distinction between the two trunks. The nerves were polyfascicular in the majority of the cases.

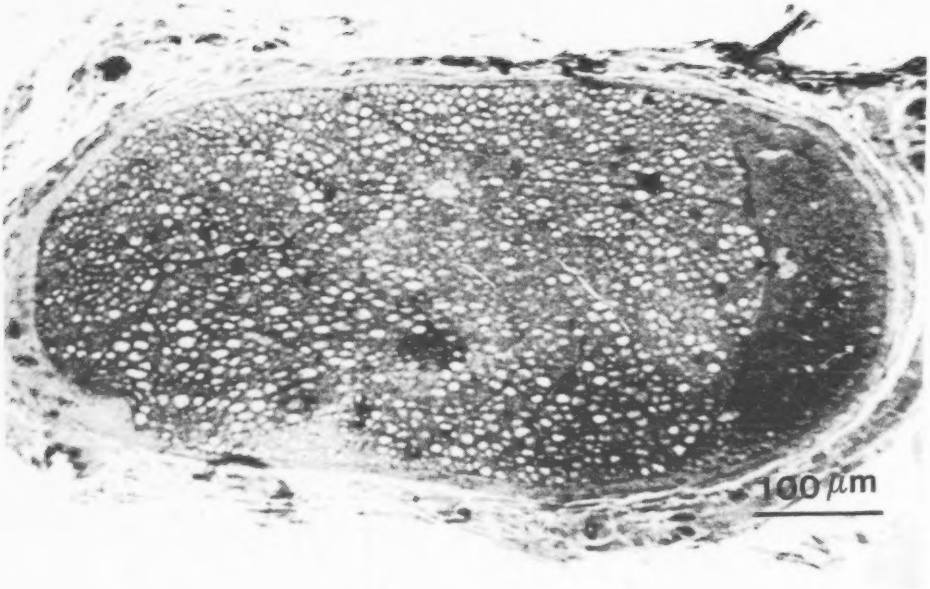


**FIGURE 11**: Transverse sections of the combined lingual and chorda tympani nerves (light micrograph, toluidine blue) distal to the transection. The only section in which it was possible to identify the lingual and the chorda tympani as independent identities distal to the transection (group I, 24 weeks, unrepaired). The chorda tympany has smaller axons and is located to the far right (✱).

## GROUP I (UNREPAIRED, 12 WEEKS SURVIVAL)

There were no statistically significant differences ($p>0.05$) in the total mean number of myelinated axons between the unoperated control (5215.67) nerve and the injured nerve at sites either proximal (4051.67) or distal (4531.33) to the injuries. Nor was there any statistically significant difference ($p>0.05$) between the counts taken proximal (4051.67) and distal (4531.33) to the injury site (Tables 3 and 4).

Proximal and distal to the transection the total mean number of myelinated axons decreased by 22% and 13% respectively (Graphic 1).

| CAT # | SECTION | UNOPERATED | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| ONE | LINGUAL | 3528 | | |
| | C. TYMPANI | 1486 | | |
| | TOTAL | 5014 | 5056 | 5797 |
| TWO | LINGUAL | 3838 | 3289 | |
| | C. TYMPANI | 1909 | 339 | |
| | TOTAL | 5747 | 3628 | 3642 |
| THREE | LINGUAL | 3507 | | |
| | C. TYMPANI | 1379 | | |
| | TOTAL | 4886 | 3471 | 4155 |

**TABLE 3:** Number of myelinated axons present in lingual, chorda tympani and their total value from sections taken proximal and distal to the injury as well as from the unoperated side in three cats from group I. The empty boxes show that in these specimen it was not possible to distinguish between the lingual and the chorda tympani nerves in some specimens.

| TOTAL MYELINATED AXON COUNTS FOR GROUP I | | | P-VALUE |
|---|---|---|---|
| CONTROL TOTAL | X | PROXIMAL TOTAL | |
| 5215.67 (464.58) | | 4051.67 (873.31) | 0.285 |
| CONTROL TOTAL | X | DISTAL TOTAL | |
| 5215.67 (464.58) | | 4531.33 (1125.71) | 0.593 |
| PROXIMAL TOTAL | X | DISTAL TOTAL | |
| 4051.67 (873.31) | | 4531.33 (1125.71) | 0.109 |

**TABLE 4:** Comparisons made concerning the number of myelinated axons from group I. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.

**GROUP II (UNREPAIRED, 24 WEEKS SURVIVAL)**

There were no statistically significant differences ($p>0.05$) in the total number of myelinated axons between the unoperated control nerve (7281.2) and the injured nerve proximal (6658.0) to the injury. Nor was there any statistically significant difference ($p>0.05$) between the counts taken proximal (4643.0) and distally (6074.8) to the injury site. Distal to the site of injury total counts of myelinated axons were reduced and the difference was statistically significant from the control ($p<0.05$) (Tables 5 and 6).

There were no significant differences ($p>0.05$) for the lingual and chorda tympani myelinated counts between proximal and control sections (Tables 5 and 6). The mean values for the lingual nerve in the control and proximal sections were 5139.0 and 4743.0 respectively. Similarly, the mean values for the chorda tympani nerve were 2142.20 and 1912.20.

Proximal and distal to the transection the total mean number of myelinated axons decreased by 9% and 17% respectively (Graphic 1). Proximal to the transection, the lingual and the chorda tympani mean number of myelinated axons decreased by 8% and 11% respectively (Graphics 2 and 3).

| CAT # | SECTION | UNOPERATED | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| 1 | LINGUAL | 5452 | 4406 | 3980 |
| | C. TYMPANI | 1876 | 2848 | 1504 |
| | TOTAL | 7328 | 7254 | 5484 |
| 2 | LINGUAL | 5878 | 5007 | |
| | C. TYMPANI | 2697 | 2098 | |
| | TOTAL | 8575 | 7105 | 7326 |
| 3 | LINGUAL | 6274 | 5367 | |
| | C. TYMPANI | 2479 | 1753 | |
| | TOTAL | 8753 | 7120 | 7550 |
| 4 | LINGUAL | 4313 | 5466 | |
| | C. TYMPANI | 1969 | 1733 | |
| | TOTAL | 6282 | 7219 | 6076 |
| 5 | LINGUAL | 3778 | 3469 | |
| | C. TYMPANI | 1690 | 1129 | |
| | TOTAL | 5468 | 4592 | 3938 |

**Table 5:** Number of myelinated axons present in the lingual, chorda tympani and their total values from control, proximal and distal sections.

| MYELINATED AXON COUNTS FROM GROUP II | | | P-VALUE |
|---|---|---|---|
| CONTROL LINGUAL<br>5139.00 (1056.75) | X | PROXIMAL LINGUAL<br>4743.00 (824.39) | 0.500 |
| CONTROL C. TYMPANI<br>2142.20 (426.20) | X | PROXIMAL C. TYMPANI<br>1912.20 (628.71) | 0.500 |
| CONTROL TOTAL<br>7281.20 (1425.52) | X | PROXIMAL TOTAL<br>6658.00 (1156.67) | 0.225 |
| CONTROL TOTAL<br>7281.20 (1425.52) | X | DISTAL TOTAL<br>6074.80 (1471.11) | 0.043 |
| PROXIMAL TOTAL<br>6658.00 (1156.67) | X | DISTAL TOTAL<br>6074.80 (1471.11) | 0.225 |

**Table 6:** Comparison made concerning the number of myelinated axons from group II. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.

### Group III (REPAIRED, 12 WEEKS SURVIVAL)

There were no statistically significant differences ($p>0.05$) in the total mean number of myelinated axons between the unoperated control nerves (5091.17) and the injured nerve at sites either proximal (5805.83) or distal (4751.0) to the injuries. However, there was significantly ($p<0.05$) more myelinated axons proximal to the injury in operated nerves than distal to it (Tables 7 and 8).

There were no statistically significant differences ($P>0.05$) in the lingual and chorda tympani counts between the unoperated control nerve and the operated nerve proximal to the injury (Tables 7 and 8). The mean values for the lingual nerve in the control and proximal sections were 3378.83 and 4297.33 respectively. Similarly, the mean values for the chorda tympani nerve were 1712.33 and 1508.50.

Proximal to the injury the total mean of myelinated axons increased by 14% and distal to it there was a decrease of 7% (Graphic 1). Proximal to the transection there was an increase of 27% for the lingual nerve and a decrease of 12% for the chorda tympani nerve (Graphics 2 and 3)

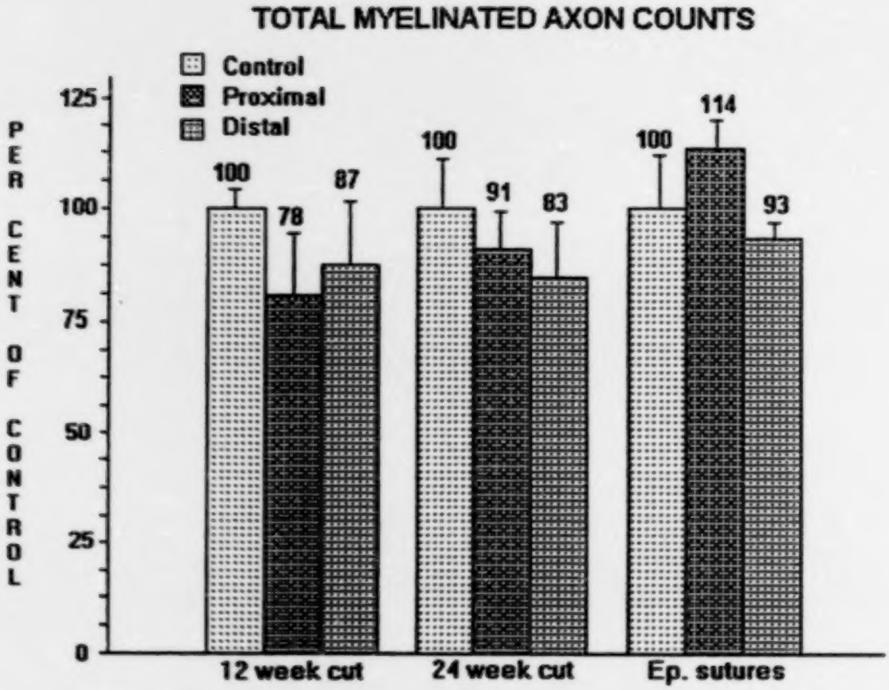
| CAT # | SECTION | UNOPERATED CONTROL | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| 1 | LINGUAL | 3872 | 3269 | |
| | C.T. | 1745 | 2366 | |
| | TOTAL | 5617 | 5635 | 4366 |
| 2 | LINGUAL | 1554 | 3741 | |
| | C.T. | 1522 | 1726 | |
| | TOTAL | 3076 | 5467 | 4743 |
| 3 | LINGUAL | 3750 | 4516 | |
| | C.T. | 1853 | 317 | |
| | TOTAL | 5603 | 4833 | 4410 |
| 4 | LINGUAL | 2258 | 3724 | |
| | C.T. | 1830 | 1533 | |
| | TOTAL | 4088 | 5257 | 4854 |
| 5 | LINGUAL | 4628 | 4969 | |
| | C.T. | 1463 | 1284 | |
| | TOTAL | 6091 | 6253 | 5309 |
| 6 | LINGUAL | 4211 | 5565 | |
| | C.T. | 1861 | 1825 | |
| | TOTAL | 6072 | 7390 | 4824 |

**Table 7:** Number of myelinated axons present in the lingual, chorda tympani and their total values from sections taken proximal and distal to the injury, as well as from the unoperated side in six cats from group III. The empty boxes show that it was not possible to distinguish between the

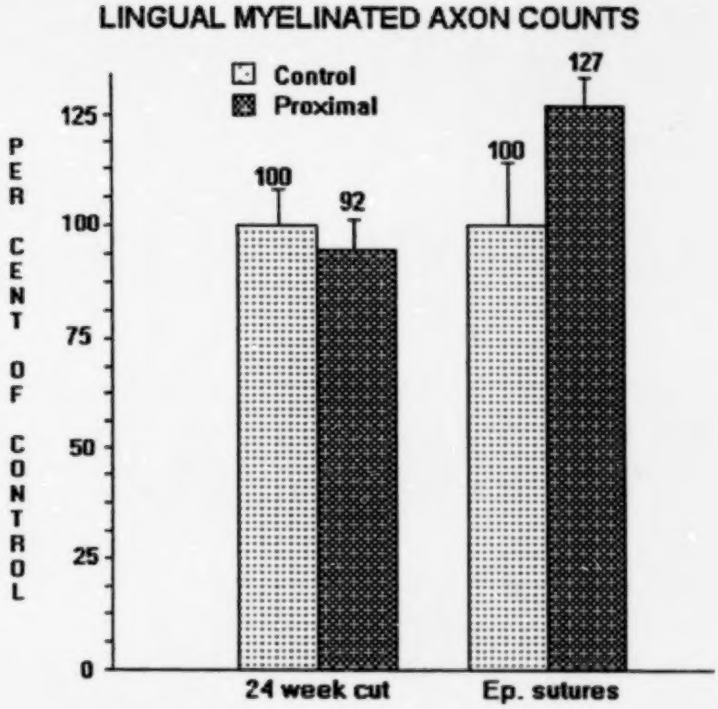
lingual and the chorda tympani nerves in some specimens.

| MYELINATED AXON COUNTS FOR GROUP III | | | P-VALUE |
|---|---|---|---|
| CONTROL LINGUAL<br>3378.83 (1201.68) | X | PROXIMAL LINGUAL<br>4297.33 (871.97) | 0.075 |
| CONTROL C. TYMPANI<br>1712.33 (176.18) | X | PROXIMAL C. TYMPANI<br>1508.50 (686.07) | 0.600 |
| CONTROL TOTAL<br>5091.17 (1230.24) | X | PROXIMAL TOTAL<br>5805.83 (905.66) | 0.116 |
| CONTROL TOTAL<br>5091.17 (1230.24) | X | DISTAL TOTAL<br>4751.00 (344.06) | 0.463 |
| PROXIMAL TOTAL<br>5805.83 (905.66) | X | DISTAL TOTAL<br>4751.00 (344.06) | 0.028 |

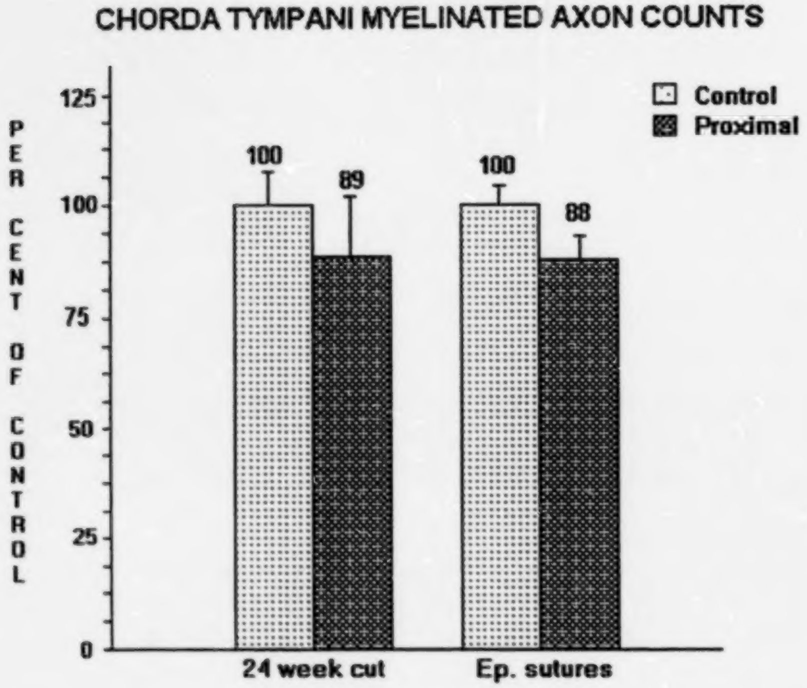
**Table 8:** Comparisons made concerning the number of myelinated axons from group III. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.



**GRAPHIC 1:** Histogram showing the percentage of the total number of myelinated axons (lingual and chorda tympani) in control, proximal and distal sections of groups I (12 weeks, unrepaired), II (24 weeks, unrepaired) and III (12 weeks repaired using epineurial sutures). Bar lines show standard errors. Fewer myelinated axons were present distal to the transection in all the groups. The only differences that were statistically significant ($p<0.05$) were between the distal and control values in group in group II (24 weeks unrepaired), and between the distal and proximal values in group III (12 weeks repaired).



**GRAPHIC 2**: Histogram showing the percentage of the lingual number of myelinated axons in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bar lines show standard errors. The lingual nerve is the major contributor for the increase seen in the total number of myelinated axons proximal to the transection in group III.



**GRAPHIC 3**: Histogram showing the percentage of the chorda tympani number of myelinated axons in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bar lines show standard error.

## NUMBER OF UNMYELINATED AXONS

Comparison for the lingual and chorda tympani nerves independently were possible only for counts taken proximal to the injury site in groups II and III. Distal to the injury site the lingual and chorda tympani nerves could not be distinguished in all the groups and only comparison of the total numbers were possible.

### GROUP I (UNREPAIRED, 12 WEEKS SURVIVAL)

There were no statistically significant differences ($p>0.05$) in the total number of unmyelinated axons between the unoperated control nerve (1995.66) and the injured nerve at sites either proximal (2942.33) or distal (9031.0) to the injuries. Nor was there any statistically significant difference ($p>0.05$) between the counts taken proximal and distal to the injury site (Tables 9 and 10).

Proximal and distal to the transection the total mean number of unmyelinated axons increased by 47% and 352% (Graphic 4).

| CAT # | SECTION | UNOPERATED CONTROL | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| 1 | LINGUAL | 1676 | | |
| | C.T. | 473 | | |
| | TOTAL | 2149 | 5040 | 15417 |
| 2 | LINGUAL | 1352 | | |
| | C.T. | 593 | | |
| | TOTAL | 1945 | 2134 | 4188 |
| 3 | LINGUAL | 1101 | | |
| | C.T. | 792 | | |
| | TOTAL | 1893 | 1653 | 7488 |

**Table 9:** Number of unmyelinated axons present in the lingual, chorda tympani and total value from sections taken proximal and distal to the injury as well as from the unoperated side in three cats from group I.

| UNMYELINATED AXON COUNT GROUP I | | | P-VALUE |
|---|---|---|---|
| CONTROL TOTAL | X | PROXIMAL TOTAL | |
| 1995.66 (135.31) | | 2942.33 (1832.48) | 0.593 |
| CONTROL TOTAL | X | DISTAL TOTAL | |
| 1995.66 (135.31) | | 9031.00 (5771.33) | 0.109 |
| PROXIMAL TOTAL | X | DISTAL TOTAL | |
| 2942.33 (1832.33) | | 9031.00 (5771.33) | 0.109 |

**Table 10:** Comparison made concerning the number of unmyelinated axons from group I. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.

**GROUP II (UNREPAIRED, 24 WEEKS SURVIVAL)**

There was no statistically significant differences (p>0.05) in the total number of unmyelinated axons between the unoperated control nerve (5480.80) and the injured nerve at sites either proximal (6038.0) or distal (5128.80) to the injuries. Nor was there any statistically significant difference (p>0.05) between the total counts taken proximal and distally to the injury (Tables 11 and 12).

There was no significant difference (p>0.05) for the lingual and chorda tympani unmyelinated counts between proximal and control sections (Tables 11 and 12). The mean values for the lingual nerve in the control and proximal sections were 3642.60 and 4053.40 respectively. Similarly, the mean values for the chorda tympani nerve were 1478.20 and 1524.20.

Proximal to the transection, the total mean number of unmyelinated axons increased by 10% and distal to it there was a decrease of 6% (Graphic 4). Proximal to the transection, the lingual and the chorda tympani mean number of unmyelinated axons increased by 24% and 3 % respectively (Graphics 5 and 6).

| CAT # | SECTION | UNOPERATED CONTROL | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| 1 | LINGUAL | 2456 | 2742 | 2726 |
|  | C.T. | 988 | 1057 | 501 |
|  | TOTAL | 3444 | 3799 | 3227 |
| 2 | LINGUAL | 2794 | 4668 |  |
|  | C.T. | 1980 | 1198 |  |
|  | TOTAL | 4774 | 5866 | 4010 |
| 3 | LINGUAL | 5709 | 4486 |  |
|  | C.T. | 1137 | 2000 |  |
|  | TOTAL | 8646 | 6486 | 6171 |
| 4 | LINGUAL | 4927 | 6285 |  |
|  | C.T. | 1622 | 1733 |  |
|  | TOTAL | 6549 | 8070 | 5877 |
| 5 | LINGUAL | 2327 | 4336 |  |
|  | C.T. | 1664 | 1633 |  |
|  | TOTAL | 3991 | 5969 | 6359 |

**Table 11:** Number of unmyelinated axons present in lingual, chorda tympani and total values from sections taken proximal and distal to the injury, as well as from the unoperated side in 5 cats from group II.

| UNMYELINATED AXON COUNTS GROUP II | | | P-VALUE |
|---|---|---|---|
| CONTROL LINGUAL | X | PROXIMAL LINGUAL | |
| 3642.60 (1563.54) | | 4503.40 (1258.18) | 0.138 |
| CONTROL C. TYMPANI | X | PROXIMAL C. TYMPANI | |
| 1478.20 (407.34) | | 1524.20 (389.39) | 0.500 |
| CONTROL TOTAL | X | PROXIMAL TOTAL | |
| 5480.80 (2122.92) | | 6038.00 (1531.11) | 0.500 |
| CONTROL TOTAL | X | DISTAL TOTAL | |
| 5480.80 (2122.92) | | 5128.80 (1416.68) | 0.345 |
| PROXIMAL TOTAL | X | DISTAL TOTAL | |
| 6038.00 (1531.11) | | 5128.80 (1416.68) | 0.138 |

**Table 12:** Comparisons made concerning the number of unmyelinated axons from group II. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.

## GROUP III (REPAIRED, 12 WEEKS SURVIVAL)

There were no statistically significant differences ($p>0.05$) in the total number of unmyelinated axons between the unoperated control nerve (4471.83) and the injured nerve proximal (6573.50) to the injury. Nor was there any statistically significant difference ($p>0.05$) between the counts taken proximal (6573.50) and distal (8137.50) to the injury site. Distal to the site of injury, total counts of unmyelinated axons were increased and the difference was statistically significant from the control ($p<0.05$) (Tables 13 and 14).

There were no significant differences ($p>0.05$) for the lingual and chorda tympani unmyelinated counts between proximal and control sections (Tables 5 and 6). The mean values for the lingual nerve in the control and proximal sections were 3244.67 and 5145.50 respectively. Similarly, the mean values for the chorda tympani nerve were 1227.17 and 1428.00.

Proximal and distal to the transection, the total mean number of unmyelinated axons increased by 46% and 82% respectively (Graphic 5). Proximal to the transection, the lingual and the chorda tympani mean number of unmyelinated axons increased by 59% and 16% respectively (Graphics 5 and 6).

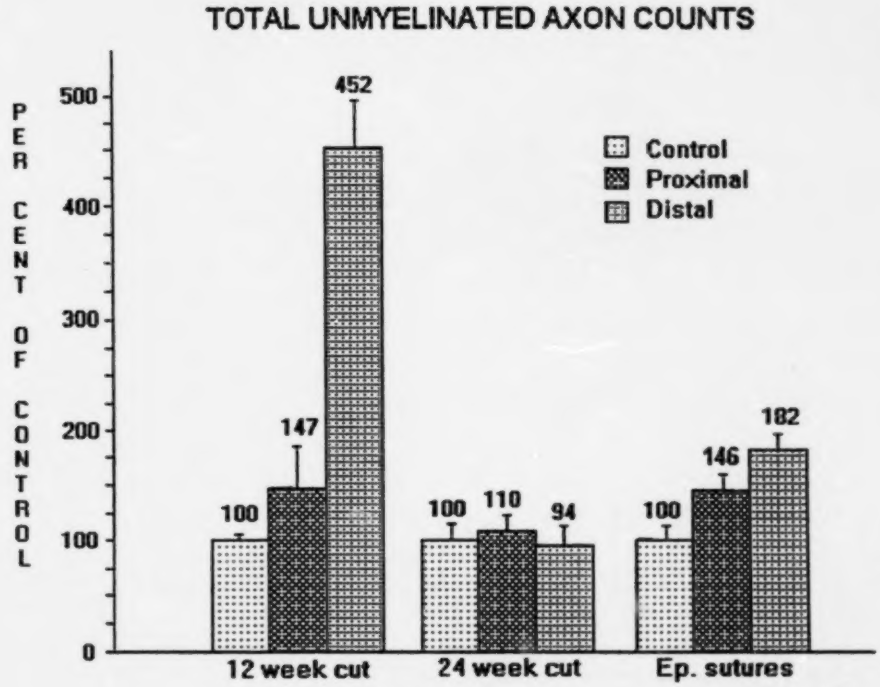
| CAT # | SECTION | UNOPERATED CONTROL | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| 1 | LINGUAL | 4731 | 4517 | |
| | C.T. | 1364 | 1345 | |
| | TOTAL | 6095 | 5862 | 10767 |
| 2 | LINGUAL | 1554 | 5965 | |
| | C.T. | 824 | 2065 | |
| | TOTAL | 2378 | 8030 | 3912 |
| 3 | LINGUAL | 3500 | 4883 | |
| | C.T. | 1324 | 285 | |
| | TOTAL | 4824 | 5168 | 10928 |
| 4 | LINGUAL | 2393 | 4160 | |
| | C.T. | 1273 | 1217 | |
| | TOTAL | 3666 | 5377 | 4854 |
| 5 | LINGUAL | 2586 | 2625 | |
| | C.T. | 1375 | 1127 | |
| | TOTAL | 3961 | 3752 | 8892 |
| 6 | LINGUAL | 4704 | 8723 | |
| | C.T. | 1203 | 2529 | |
| | TOTAL | 5907 | 11252 | 9472 |

**Table 13:** Number of unmyelinated axons present in lingual,

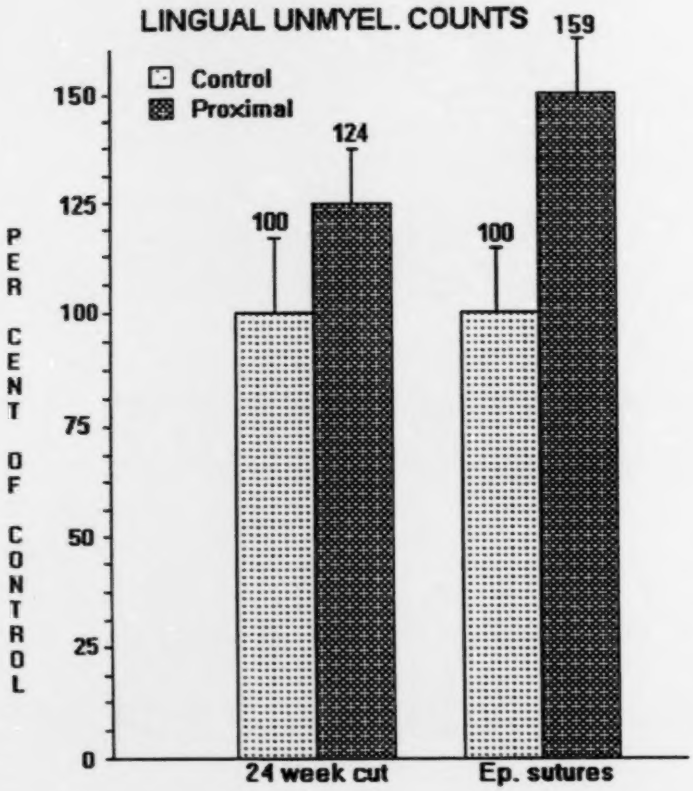
chorda tympani and total values from sections taken proximal and distal to the injury as well as from the unoperated side in six cats from group III.

| UNMYELINATED AXON COUNT GROUP III | | | P-VALUE |
|---|---|---|---|
| CONTROL LINGUAL | X | PROXIMAL LINGUAL | |
| 3244.67 (1297.81) | | 5145.50 (2061.95) | 0.075 |
| CONTROL C. TYMPANI | X | PROXIMAL C. TYMPANI | |
| 1227.17 (207.45) | | 1428.00 (783.13) | 0.917 |
| CONTROL TOTAL | X | PROXIMAL TOTAL | |
| 4471.83 (1422.17) | | 6573.50 (2678.95) | 0.116 |
| CONTROL TOTAL | X | DISTAL TOTAL | |
| 4471.83 (1422.17) | | 8137.50 (3022.83) | 0.028 |
| PROXIMAL TOTAL | X | DISTAL TOTAL | |
| 6573.50 (2678.95) | | 8137.50 (3022.83) | 0.345 |

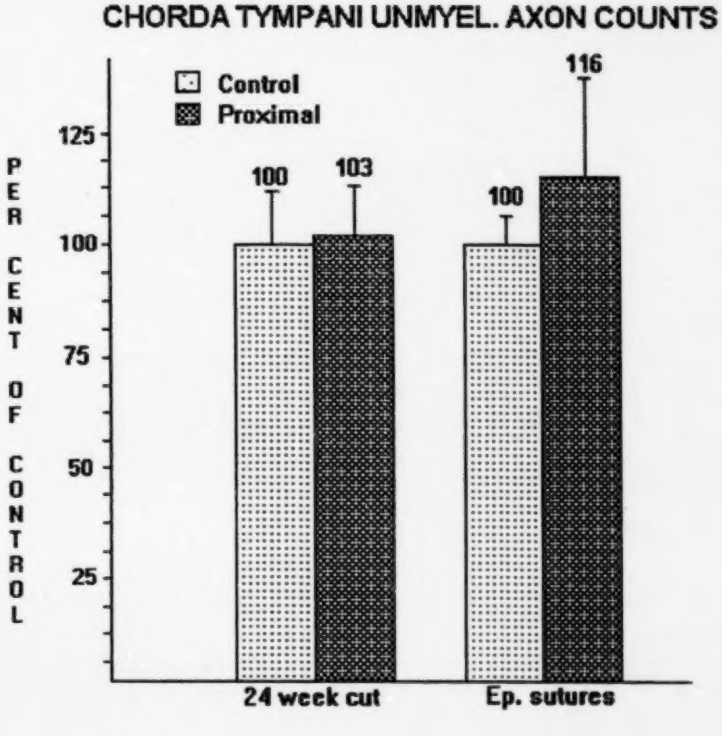
**Table 14:** Comparisons made concerning the number of unmyelinated for group III. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.



**GRAPHIC 4:** A histogram showing the percentage of the total number of unmyelinated axons (lingual and chorda tympani) in control, proximal and distal sections of groups I (12 weeks, unrepaired), II (24 weeks, unrepaired) and III (12 weeks repaired using epineurial sutures). Bar lines show standard errors. More unmyelinated axons were present proximal and distal to the transection in the groups that had 12 weeks recovery time (Groups I and II). The only difference that was statistically significant was between distal and control values in group III.



**GRAPHIC 5**: Histogram showing the percentage of the lingual number of unmyelinated axons in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bar lines show standard errors. The lingual nerve is the major contributor for the increase seen in the total number of unmyelinated axons proximal to the transection in groups II and III.



**GRAPHIC 6**: Histogram showing the percentage of the chorda tympani number of unmyelinated axons in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). The vertical bars indicate the deviations (Standard Error) from these numbers.

## MYELINATED AXON SIZE

Myelinated axon size refers to the perimeter of the axons encountered in the analyzed sites of the nerve trunk.

## GROUP I (UNREPAIRED, 12 WEEKS SURVIVAL)

There were no statistically significant differences ($P>0.05$) in the total mean myelinated axonal sizes between the unoperated control nerve (17.76) and the injured nerve at sites either proximal (22.46) or distal (12.95) to the injuries. Nor was there any statistically significant difference ($p>0.05$) between values taken proximal and distally to the injury site (Tables 15 and 16). The myelinated axons are approximately of the same size in unoperated nerves and in operated nerves proximal to the transection (Figure 12). Figure 13 shows a reduction in the myelinated axonal size distal to the transection.

Proximal to the transection there was an increase in the total mean myelinated axonal size of 29%. Distal to the transection there was a decrease of 27% (Graphic 7).

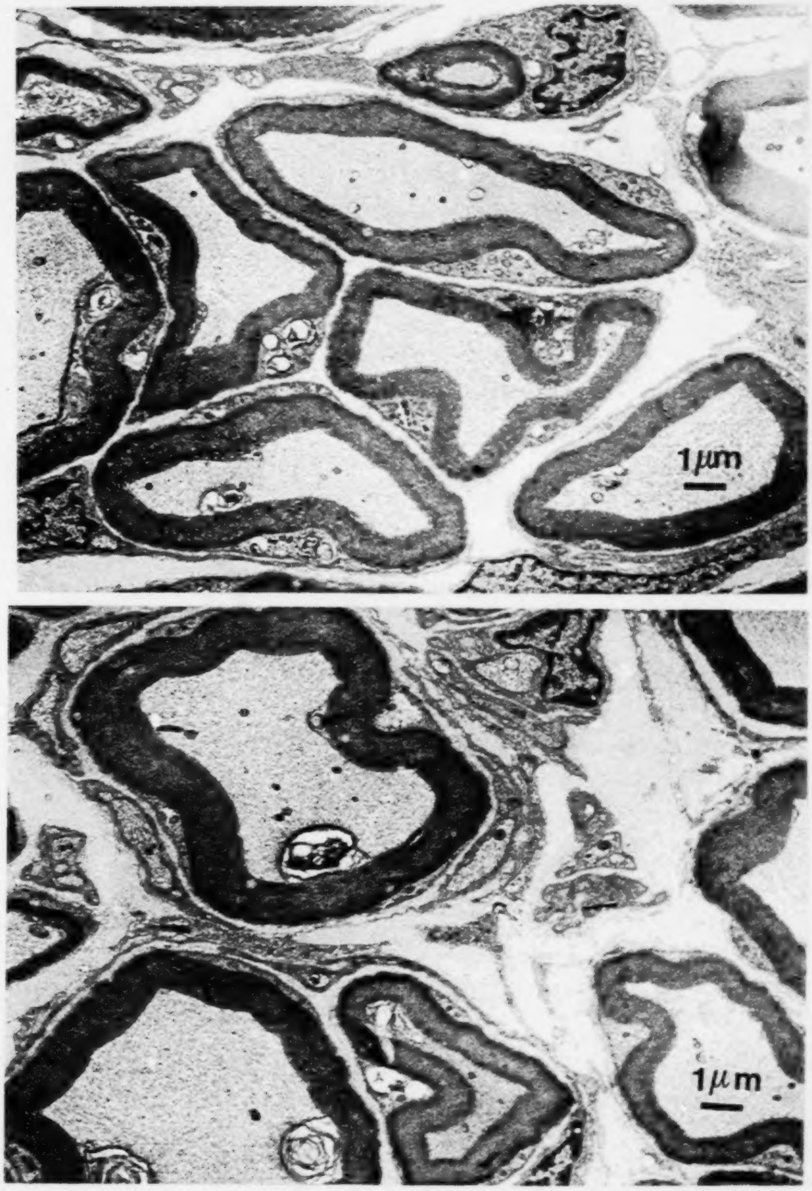


**FIGURE 12**: Transverse sections of the lingual nerve (electron micrographs, uranyl acetate and lead citrate). The upper micrograph is from the control side, the lower is from proximal to the transection of group I (unrepaired, 12 weeks). The myelinated axons have approximately the size in the control and proximal sections. The thickness of their myelin sheath is also similar.

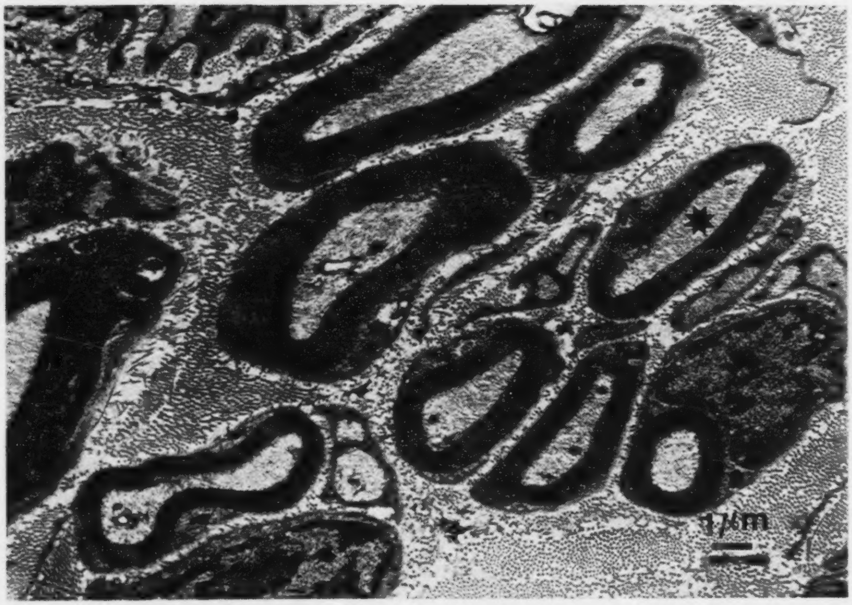


**FIGURE 13**: Transverse sections of the lingual nerve distal to the transection (electron micrograph, uranyl acetate and lead citrate) from group I (unrepaired, 12 weeks). In this group only the lingual axons were able to cross the injury site. The myelinated axons are smaller (✱) and have thinner myelin sheaths than that of control and proximal sections.

| # | SECTION | UNOPERATED CONTROL (μm) | OPERATED PROXIMAL (μm) | OPERATED DISTAL (μm) |
|---|---|---|---|---|
| 1 | LINGUAL | 24.38 (11.07) | | |
| | C.T. | 16.94 (5.94) | | |
| | TOTAL | 22.10 (10.31) | 23.91 (10.43) | 12.79 (4.37) |
| 2 | LINGUAL | 18.93 (8.95) | | |
| | C.T. | 12.37 (3.35) | | |
| | TOTAL | 15.65 (7.51) | 22.66 (10.63) | 12.24 (4.45) |
| 3 | LINGUAL | 18.34 (9.09) | | |
| | C.T. | 11.61 (3.06) | | |
| | TOTAL | 15.53 (7.94) | 20.80 (8.92) | 13.82 (5.06) |

**Table 15:** Myelinated axonal size from lingual, chorda tympani and total values in three cats from group I. Proximal, distal and control sites were analyzed. The empty boxes show that it was not possible to distinguish between the lingual and the chorda tympani nerves in some specimens. Obs.: The values are Means and Standard Deviation (S.D.).

| MYELINATED AXON SIZE FOR GROUP I | | | P-VALUE |
|---|---|---|---|
| CONTROL TOTAL (μm) | X | PROXIMAL TOTAL (μm) | |
| 17.76 (3.76) | | 22.46 (1.56) | 0.109 |
| CONTROL TOTAL (μm) | X | DISTAL TOTAL (μm) | |
| 17.76 (3.76) | | 12.95 (0.80) | 0.109 |
| PROXIMAL TOTAL (μm) | X | DISTAL TOTAL (μm) | |
| 22.46 (1.56) | | 12.95 (0.80) | 0.109 |

**Table 16:** Comparisons made concerning the myelinated axonal size from group I. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.

**GROUP II (UNREPAIRED, 24 WEEKS)**

There were no statistically significant differences ($p>0.05$) in the total mean myelinated axonal sizes between the unoperated control nerve (18.26) and the injured nerve at sites either proximal (18.56) or distal (15.41) to the injuries. Nor was there any statistically significant difference ($p>0.05$) between values taken proximal (18.56) and distally (15.41) to the injury site (Tables 17 and 18). Figures 14 and 15 show that the lingual and the chorda tympani myelinated axons are of approximately the same size in unoperated nerves and in operated nerves proximal to the transection. Figure 16 shows that the myelinated axons present distal to the transection are smaller than the myelinated axons found in the lingual nerve.

There were no significant differences ($p>0.05$) for the lingual and chorda tympani mean myelinated axonal size between proximal and control sections (Tables 17 and 18). The mean values for the lingual nerve in the control and proximal sections were 20.64 and 20.88 respectively. Similarly, the mean values for the chorda tympani nerve were 13.65 and 14.73.

Proximal to the transection the total mean myelinated axonal size increased by 2%. Distal to the transection the total mean myelinated axonal size decreased by 16% (Graphic 7). Proximal to the transection the lingual and the chorda tympani nerve mean myelinated axonal size increased by 1% and

8% respectively (Graphics 8 and 9).

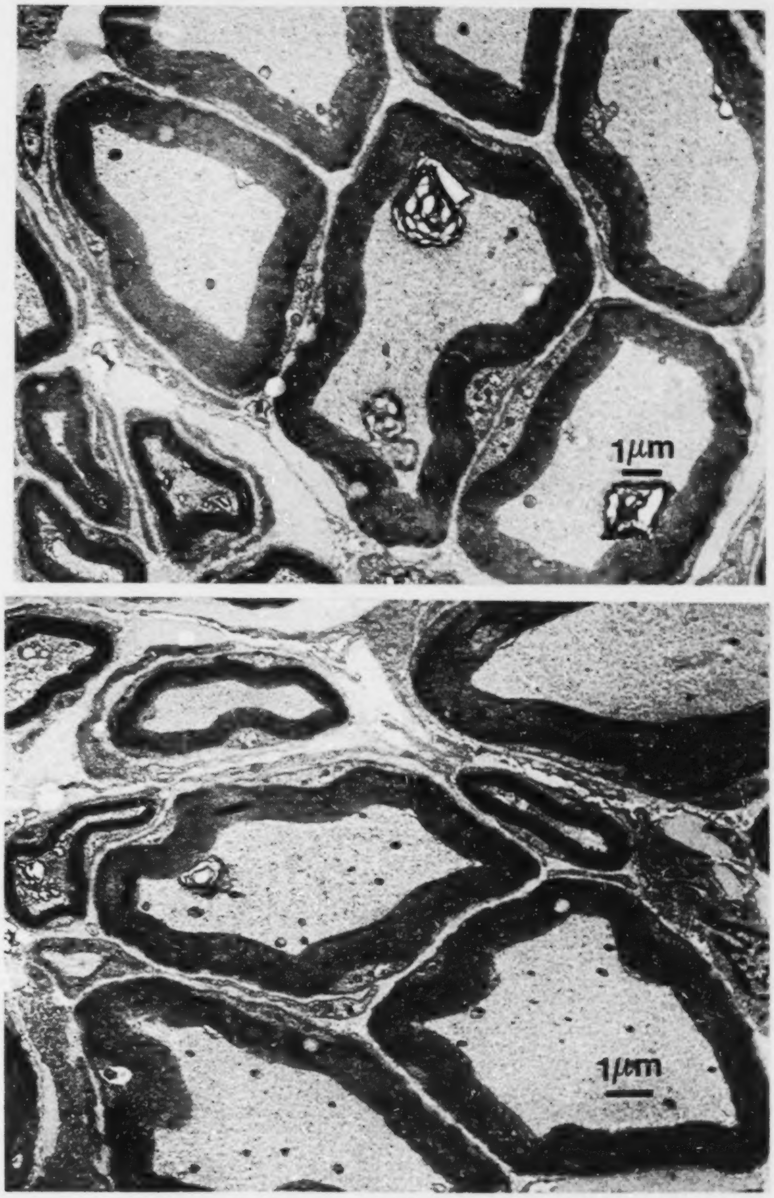


**FIGURE 14**: Transverse sections of the lingual nerves (electron micrographs, uranyl acetate and lead citrate). The upper micrograph is from the control side, the lower is from proximal to the transection of group II (unrepaired, 24 weeks). The myelinated axons have approximately the same size in control and proximal sections. The thickness of their myelin is also similar.

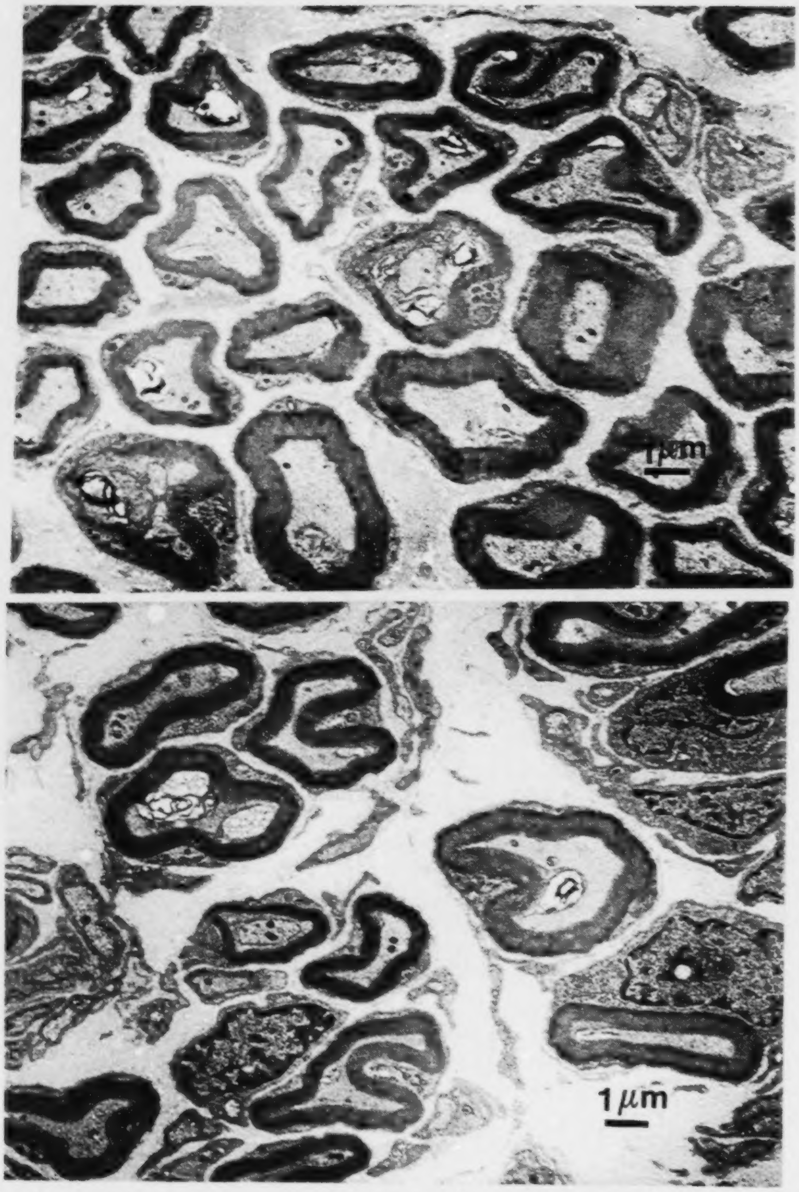


**FIGURE 15**: Transverse sections of the chorda tympani nerve (electron micrograph, uranyl acetate and lead citrate). The upper micrograph is from the control side, the lower proximal to the transection of group II (unrepaired, 24 weeks). The chorda tympani axons are smaller than the lingual axons. The myelinated axons from the control and proximal sections have approximately the same size and the thickness of their myelin sheaths is also similar.

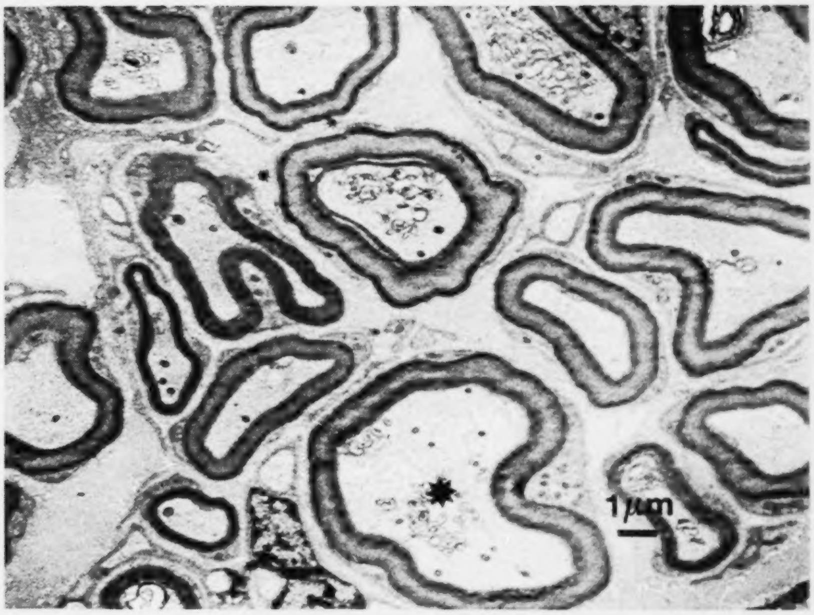


**FIGURE 16**: Transverse sections of the lingual nerve (electron micrograph, uranyl acetate, lead citrate). This micrograph is from distal to the transection from group II (unrepaired, 24 weeks). Distal to the transection it was not possible to identify the lingual and the chorda tympani as separate nerve trunks. The myelinated axons are smaller (✱) and have thinner myelin sheath than the lingual myelinated axons from the control and proximal sections.

| # | SECTION | UNOPERATED CONTROL (µm) | OPERATED PROXIMAL (µm) | OPERATED DISTAL (µm) |
|---|---|---|---|---|
| 1 | LINGUAL | 18.11 (8.61) | 20.83 (10.69) | 21.60 (10.02) |
|  | C.T. | 13.13 (5.20) | 12.02 (3.12) | 13.95 (4.11) |
|  | TOTAL | 16.58 (8.06) | 16.93 (9.33) | 18.71 (9.08) |
| 2 | LINGUAL | 20.41 (8.79) | 20.04 (8.66) |  |
|  | C.T. | 13.18 (3.38) | 14.16 (4.00) |  |
|  | TOTAL | 17.93 (8.15) | 18.35 (8.07) | 13.75 (5.56) |
| 3 | LINGUAL | 19.03 (13.50) | 19.96 (9.29) |  |
|  | C.T. | 12.33 (3.30) | 13.05 (3.53) |  |
|  | TOTAL | 16.65 (11.47) | 15.69 (6.03) | 15.44 (6.56) |
| 4 | LINGUAL | 22.50 (10.24) | 22.43 (11.85) |  |
|  | C.T. | 14.73 (8.52) | 15.29 (4.21) |  |
|  | TOTAL | 19.98 (10.36) | 21.19 (11.24) | 14.54 (6.01) |
| 5 | LINGUAL | 23.16 (10.81) | 21.15 (9.68) |  |
|  | C.T. | 14.87 (3.55) | 19.13 (5.88) |  |
|  | TOTAL | 20.14 (9.73) | 20.64 (8.92) | 14.63 (6.26) |

**Table 17:** Myelinated axonal size from lingual, chorda tympani and total values in five cats of group II. Proximal, distal and control sites were analyzed. Obs: The values are Means and Standard deviation (S.D.).

| MYELINATED AXONAL SIZE FROM GROUP II | | | P-VALUE |
|---|---|---|---|
| CONTROL LINGUAL (µm) | X | PROXIMAL LINGUAL (µm) | |
| 20.64 (2.17) | | 20.88 (1.00) | 0.893 |
| CONTROL C.T. (µm) | X | PROXIMAL C.T. (µm) | |
| 13.65 (1.11) | | 14.73 (2.75) | 0.345 |
| CONTROL TOTAL (µm) | X | PROXIMAL TOTAL (µm) | |
| 18.26 (1.73) | | 18.56 (2.35) | 0.345 |
| CONTROL TOTAL (µm) | X | CONTROL DISTAL (µm) | |
| 18.26 (1.73) | | 15.41 (1.94) | 0.138 |
| PROXIMAL TOTAL (µm) | X | DISTAL TOTAL (µm) | |
| 18.56 (2.35) | | 15.41 (1.94) | 0.138 |

**Table 18:** Comparisons made concerning the myelinated axonal size from group II. Obs.: The values represent Means, Standard Deviation (S.D.) and P-value.

**GROUP III (REPAIRED, 12 WEEKS)**

There were no statistically significant differences (P>0.05) in the total mean myelinated axonal sizes between the unoperated control nerve (19.94) and the injured nerve proximal (20.35) to the injury. Distal to the site of injury the total mean myelinated axonal size was reduced (14.94) and the difference was statistically significant (p<0.05) from both control (19.94) and proximal values (20.35)(Tables 19 and 20). Figures 17 and 18 show that the lingual and the chorda tympani myelinated axons are of approximately the same size in unoperated nerves and in operated nerves proximal to the transection. Figure 19 shows that the myelinated axons present distal to the transection are smaller than the myelinated axons found in the lingual nerve.

There was no significant difference (p>0.05) for the lingual and chorda tympani mean myelinated axonal size between proximal and control sections (Tables 19 and 20) (Figures 17 and 18). The mean values for the lingual nerve in the control and proximal sections were 22.76 and 23.23 respectively. Similarly, the mean values for the chorda tympani nerve were 13.97 and 14.31.

Proximal to the transection, the total mean myelinated axonal size increased by 2%. Distal to the transection the total mean myelinated axonal size decreased by 25% (Graphic 7). Proximal to the transection the lingual and the chorda tympani nerve mean myelinated axonal size increased by 2%

(Graphics 8 and 9).

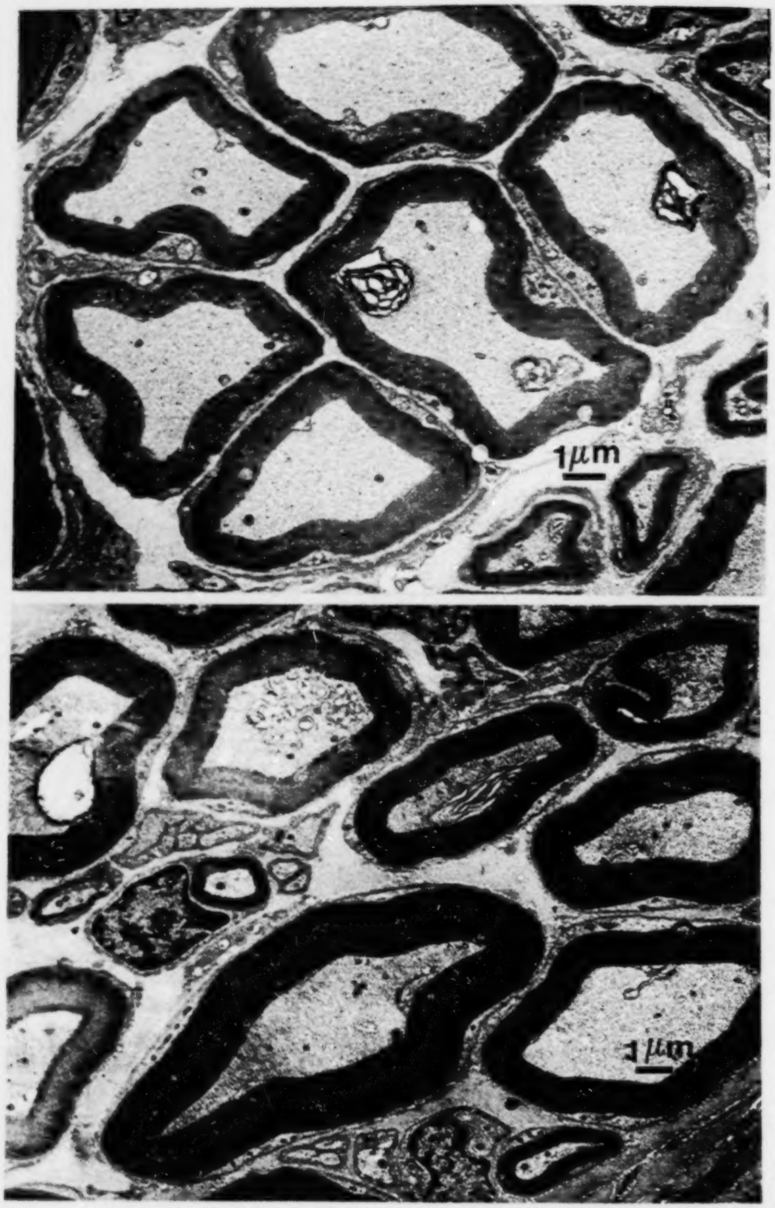


**FIGURE 17**: Transverse sections of the lingual nerve (electron micrographs, uranyl acetate and lead citrate). The upper micrograph is from the control side, the lower from proximal to the transection of group III (repaired, 12 weeks). The myelinated axons have approximately the same size in control and proximal sections. The thickness of their myelin sheath is also similar.

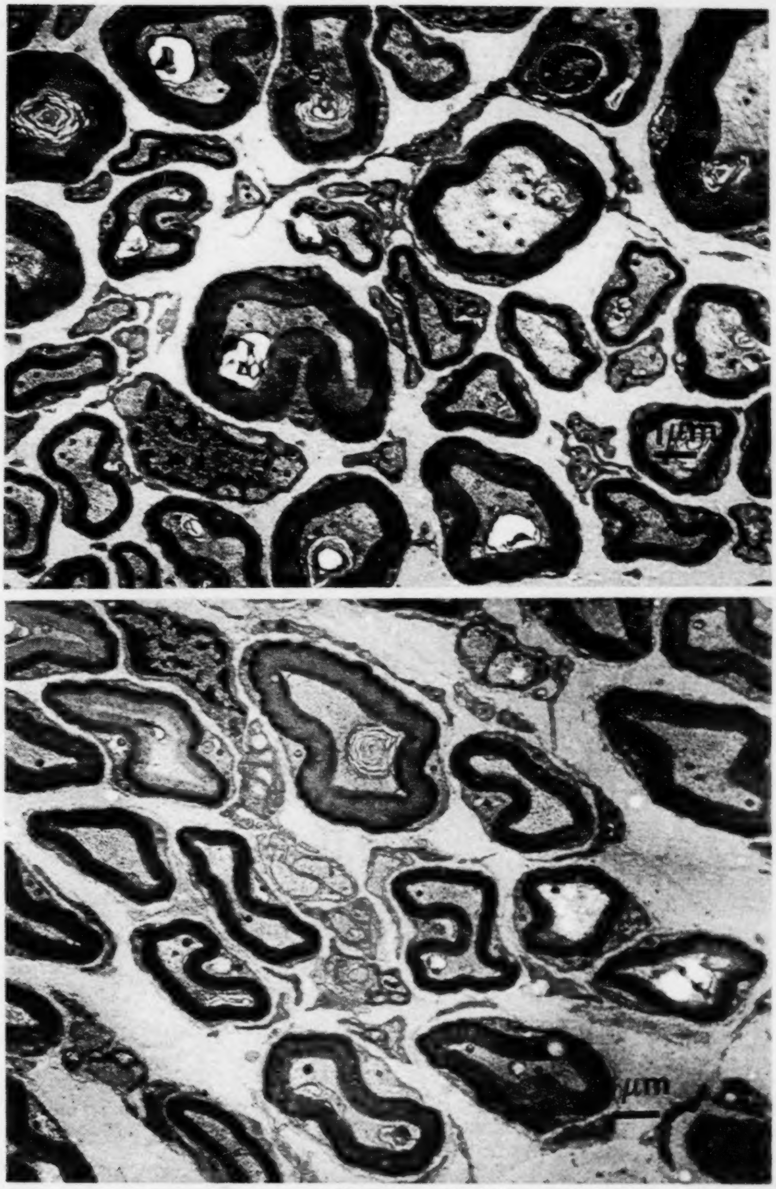


**FIGURE 18**: Transverse sections of the chorda tympani nerve (electron micrographs, uranyl acetate and lead citrate). The upper micrograph is from the control side, the lower from the proximal to the transection. The chorda tympani axons are smaller than the lingual axons. The myelinated axons have approximately the same size in control and proximal sections. The thickness of their myelin sheaths is also similar.

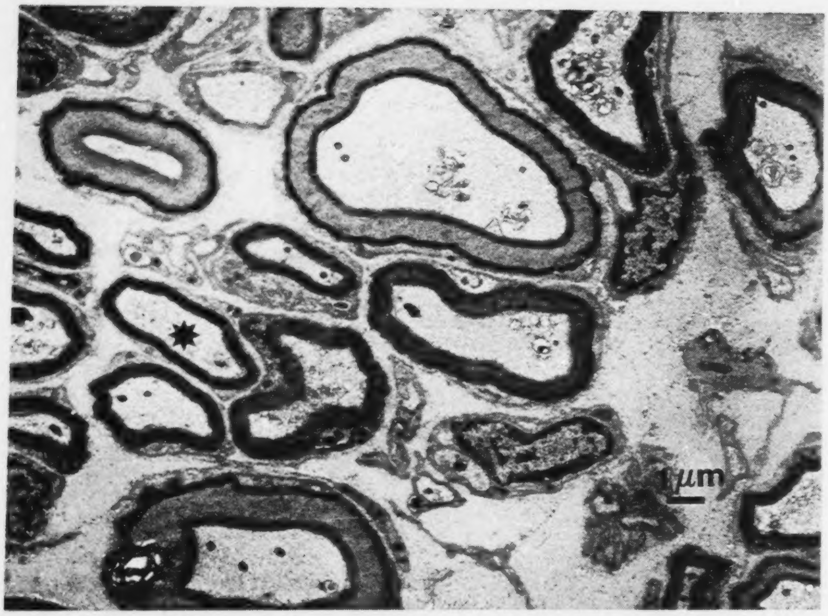


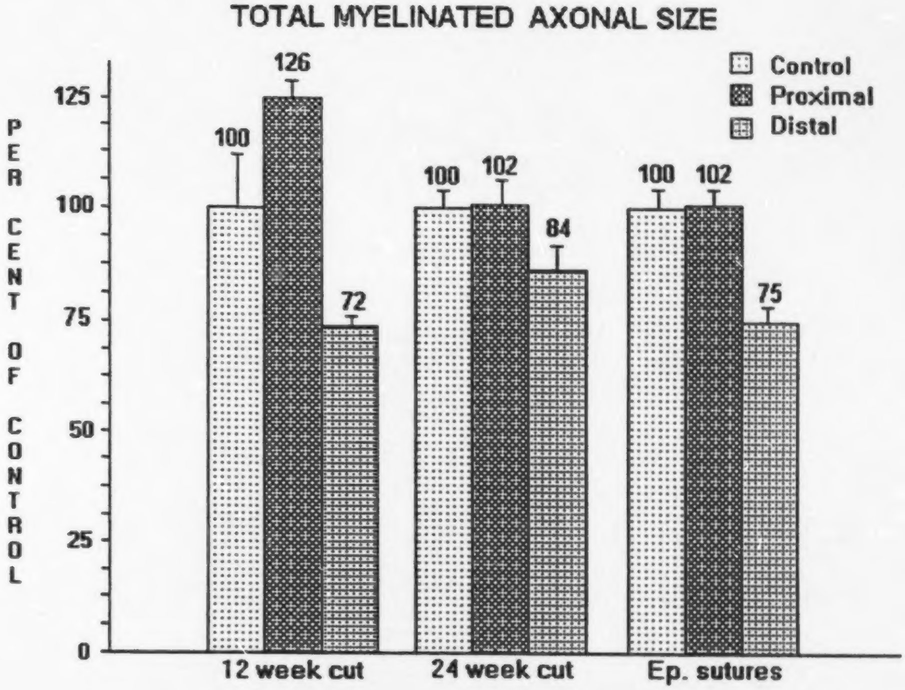
**FIGURE 19**: Electron micrograph of the lingual nerve distal to the transection from group III (repaired, 12 weeks). Distal to the transection it was not possible to identify the lingual and the chorda tympani as separate nerve trunks. The myelinated axons are smaller and have thinner myelin sheath (✱)than the lingual myelinated axons from the control and proximal sections.

| # | SECTION | UNOPERATED CONTROL (µm) | OPERATED PROXIMAL (µm) | OPERATED DISTAL (µm) |
|---|---|---|---|---|
| 1 | LINGUAL | 21.45 (10.00) | 24.85 (11.66) | |
| | C.T. | 12.10 (3.79) | 13.08 (3.55) | |
| | TOTAL | 18.63 (9.62) | 19.53 (10.69) | 14.73 (5.79) |
| 2 | LINGUAL | 21.26 (9.17) | 26.42 (12.75) | |
| | C.T. | 13.21 (3.47) | 13.61 (3.96) | |
| | TOTAL | 17.85 (8.33) | 22.29 (12.96) | 12.72 (4.60) |
| 3 | LINGUAL | 23.78 (10.37) | 18.39 (7.94) | |
| | C.T. | 13.69 (3.81) | 15.34 (6.57) | |
| | TOTAL | 20.15 (9.87) | 17.96 (7.83) | 12.20 (4.70) |
| 4 | LINGUAL | 26.65 (11.89) | 24.13 (11.61) | |
| | C.T. | 15.40 (4.97) | 16.75 (4.39) | |
| | TOTAL | 24.86 (11.82) | 22.27 (10.76) | 14.45 (4.55) |
| 5 | LINGUAL | 20.94 (8.92) | 25.28 (12.20) | |
| | C.T. | 15.11 (4.79) | 13.77 (3.13) | |
| | TOTAL | 19.40 (8.44) | 21.62 (11.15) | 15.36 (5.93) |
| 6 | LINGUAL | 22.46 (10.39) | 20.28 (8.87) | |
| | C.T. | 14.32 (3.89) | 12.99 (3.62) | |
| | TOTAL | 18.74 (9.05) | 18.45 (8.49) | 16.17 (6.76) |

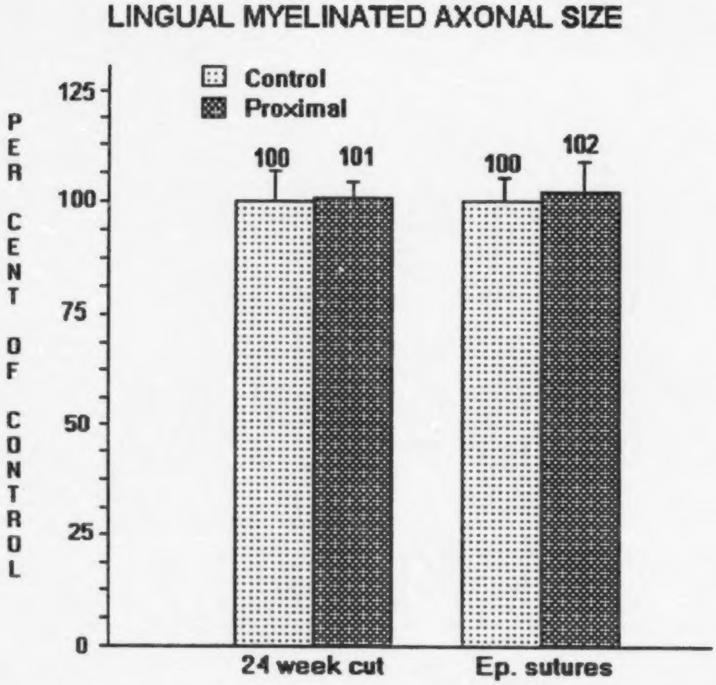
**Table 19:** Myelinated axonal size from lingual, chorda tympani and total values in six cats from group III. Proximal, distal and control sites were analyzed. Obs: The values are Means and Standard Deviation (S.D.).

| MYELINATED AXON SIZE FOR GROUP III | | P-VALUE |
|---|---|---|
| CONTROL LINGUAL (μm) X | PROXIMAL LINGUAL (μm) | |
| 22.76 (2.17) | 23.23 (3.16) | 0.893 |
| CONTROL C.T. (μm) X | PROXIMAL C.T. (μm) | |
| 13.97 (1.23) | 14.31 (1.53) | 0.345 |
| CONTROL TOTAL (μm) X | PROXIMAL TOTAL (μm) | |
| 19.94 (2.53) | 20.35 (1.95) | 0.893 |
| CONTROL TOTAL (μm) X | DISTAL TOTAL (μm) | |
| 19.94 (2.53) | 14.94 (1.53) | 0.028 |
| PROXIMAL TOTAL (μm) X | DISTAL TOTAL (μm) | |
| 20.35 (1.95) | 14.94 (1.53) | 0.028 |

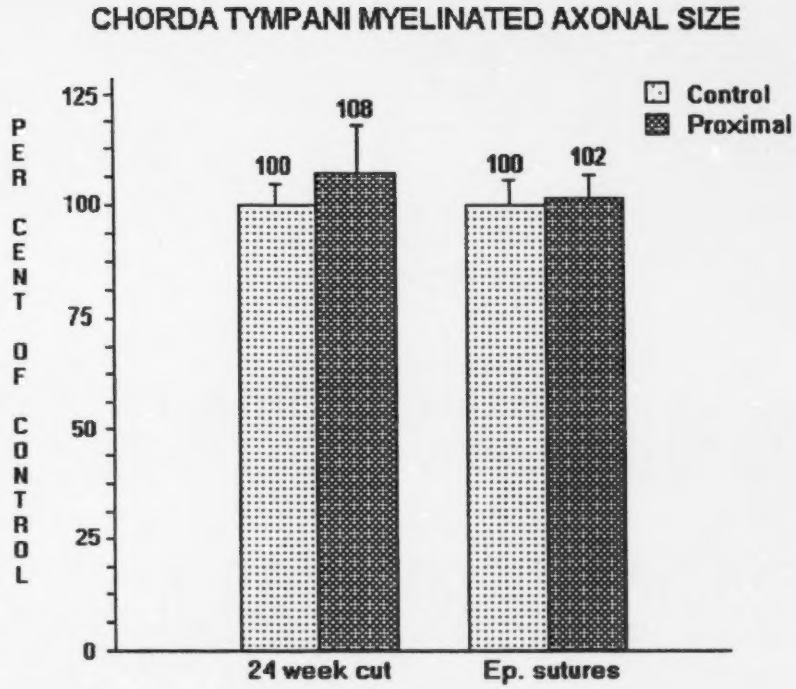
**Table 20:** Comparisons made concerning the myelinated axonal size from group I. Obs: The values represent Means, Standard Deviation (S.D.) and P-value.



**GRAPHIC 7:** Histogram showing the percentage of the total myelinated axonal size (lingual and chorda tympani) in control, proximal and distal sections of groups I (12 weeks, unrepaired), II (24 weeks, unrepaired) and III (12 weeks repaired using epineurial sutures). Bar lines show standard errors. The myelinated axons are reduced distal to the transection. The only difference that was statistically significant (p<0,05) was between the control and proximal values group III.



**GRAPHIC 8**: Histogram showing the percentage of lingual myelinated axonal size in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bars lines show standard error.



**GRAPHIC 9**: Histogram showing the percentage of chorda tympani myelinated axonal size in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bar lines show standard errors. The chorda tympani is the major contributor for the increase seen in total myelinated axonal size in group II.

## THICKNESS OF THE MYELIN SHEATH

### GROUP I (UNREPAIRED, 12 WEEKS SURVIVAL)

There were no statistically significant differences ($p>0.05$) in the total mean thickness of the myelin sheaths between the unoperated control nerve (0.83) or the injured nerve at sites either proximal (1.07) or distal (0.61) to the injuries. Nor was there any statistically significant difference ($p>0.05$) between values taken proximal (1.07) and distally (0.61)to the injury site (Tables 21 and 22). Proximal to the transection the total mean thickness of the myelin sheath increased by 29%. Distal to the transection the total thickness of the myelin sheath decreased by 27% (Graphic 10)

| CAT # | SECTION | UNOPERATED CONTROL (μm) | OPERATED PROXIMAL (μm) | OPERATED DISTAL (μm) |
|---|---|---|---|---|
| 1 | LINGUAL | 1.23 (0.04) | | |
| | C.T. | 0.84 (0.03) | | |
| | TOTAL | 1.11 (0.03) | 1.01 (0.45) | 0.54 (0.17) |
| 2 | LINGUAL | 0.84 (0.03) | | |
| | C.T. | 0.51 (0.01) | | |
| | TOTAL | 0.68 (0.02) | 1.05 (0.58) | 0.58 (0.18 |
| 3 | LINGUAL | 0.84 (0.03) | | |
| | C.T. | 0.49 (0.01) | | |
| | TOTAL | 0.69 (0.02) | 1.15 (0.52) | 0.70 (0.23) |

**Table 21:** Thickness of the myelin sheath from lingual, chorda tympani and total values in three cats from group I. Proximal, distal and control sites were analyzed. Obs.: The values are Means and Standard Deviation (S.D.).

| THICKNESS OF THE MYELIN SHEATH FROM GROUP I | | | P-VALUE |
|---|---|---|---|
| CONTROL TOTAL (μm) | X | PROXIMAL TOTAL (μm) | |
| 0.83 (0.25) | | 1.07 (1.07) | 0.109 |
| CONTROL TOTAL (μm) | X | DISTAL TOTAL (μm) | |
| 0.83 (0.25) | | 0.61 (0.08) | 1.000 |
| PROXIMAL TOTAL (μm) | X | DISTAL TOTAL (μm) | |
| 1.07 (0.07) | | 0.61 (0.08) | 0.109 |

**Table 22:** Comparisons made concerning the thickness of the myelin sheath from group I. Obs.: The values represent Means, Standard Deviation (S.D.) and P-value.

**GROUP II (UNREPAIRED, 24 WEEKS SURVIVAL)**

There were no statistically significant differences (p>0.05) in the total mean thickness of the myelin sheaths between the unoperated control nerve (0.91) and the injured nerve at sites either proximal (0.85) or distal (0.71) to the injuries. Nor was there any statistically significant difference (p>0.05) between values taken proximal (0.85) and distal (0.71) to the injury site (Tables 23 and 24).

There were no significant differences (p>0.05) for the lingual and chorda tympani mean thickness of the myelin sheath between proximal and control sections (Tables 23 and 24). The mean values for the lingual nerve in the control and proximal sections were 1.06 and 0.92. Similarly, the mean values for the chorda tympani nerve were 0.63 and 0.69.

Proximal and distal to the transection the mean thickness of the myelin sheath decreased by 7% and 22% respectively (Graphic 10). Proximal to the transection the lingual mean thickness of the myelin sheath decreased by 14% (Graphic 11). The chorda tympani mean thickness of the myelin sheath increased by 9% (Graphic 12).

| CAT # | SECTION | UNOPERATED CONTROL (μm) | OPERATED PROXIMAL (μm) | OPERATED DISTAL (μm) |
|---|---|---|---|---|
| 1 | LINGUAL | 0.88 (0.44) | 1.08 (0.58) | 0.95 (0.48) |
| | C.T. | 0.60 (0.28) | 0.63 (0.21) | 0.67 (0.24) |
| | TOTAL | 0.79 (0.41) | 0.88 (0.51) | 0.84 (0.43) |
| 2 | LINGUAL | 1.16 (0.49) | 0.84 (0.36) | |
| | C.T. | 0.69 (0.22) | 0.65 (0.24) | |
| | TOTAL | 1.00 (0.47) | 0.79 (0.35) | 0.58 (0.20) |
| 3 | LINGUAL | 0.90 (0.44) | 0.83 (0.31) | |
| | C.T. | 0.53 (0.16) | 0.57 (0.18) | |
| | TOTAL | 0.77 (0.41) | 0.76 (0.30) | 0.73 (0.26) |
| 4 | LINGUAL | 1.12 (0.52) | 0.99 (0.57) | |
| | C.T. | 0.64 (0.22) | 0.77 (0.27) | |
| | TOTAL | 0.96 (0.50) | 0.95 (0.54) | 0.66 (0.23) |
| 5 | LINGUAL | 1.22 (0.56) | 0.85 (0.39) | |
| | C.T. | 0.70 (0.25) | 0.84 (0.30) | |
| | TOTAL | 1.03 (0.53) | 0.85 (0.37) | 0.73 (0.08) |

**Table 23:** Thickness of the myelin sheath for lingual, chorda tympani and total values from sections taken proximal and distally to the injury as well as from the unoperated side in five cats from group II. Obs.: The values are Means and Standard Deviation (S.D.).

| THICKNESS OF THE MYELIN SHEATH FOR GROUP II | | | P-VALUE |
|---|---|---|---|
| CONTROL LINGUAL (μm) | X | PROXIMAL LINGUAL (μm) | |
| 1.06 (0.16) | | 0.92 (0.11) | 0.225 |
| CONTROL C.T. (μm) | X | PROXIMAL C.T. (μm) | |
| 0.63 (0.07) | | 0.69 (0.11) | 0.178 |
| CONTROL TOTAL (μm) | X | PROXIMAL TOTAL (μm) | |
| 0.91 (0.12) | | 0.85 (0.08) | 0.225 |
| CONTROL TOTAL (μm) | X | DISTAL TOTAL (μm) | |
| 0.91 (0.12) | | 0.71 (0.10) | 0.138 |
| PROXIMAL TOTAL (μm) | X | DISTAL TOTAL (μm) | |
| 0.85 (0.08) | | 0.71 (0.10) | 0.138 |

**Table 24:** Comparisons made concerning the thickness of the myelin sheath from group II. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.

### GROUP III (REPAIRED, 12 WEEKS)

There were no statistically significant differences ($P>0.05$) in the total mean thickness of the myelin sheaths between the unoperated control nerves (1.12) and the injured nerves proximal (1.00) to the injury. Distal to the site of injury the total mean thickness of the myelin sheath was reduced (0.67) and the difference was statistically significant ($p<0.05$) from both control (1.12) and proximal values (1.00)(Tables 25 and 26).

There were no significant differences ($p>0.05$) for the lingual and chorda tympani mean thickness of the myelin sheath between proximal and control sections (Tables 25 and 26). The mean values for the lingual nerve in the control and proximal sections were 1.32 and 1.14 respectively. Similarly, the mean values for the chorda tympani nerve were 0.70 and 0.69.

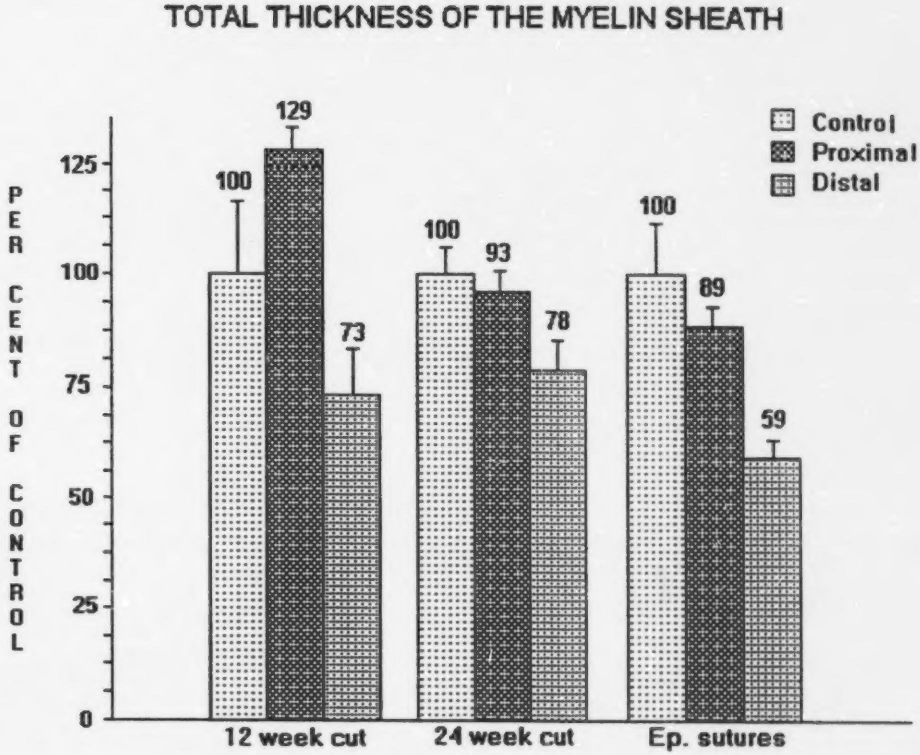
Proximal and distal to the injury the total mean thickness of the myelin sheath decreased by 11% and 41% respectively (Graphic 10). Proximal to the injury the lingual and the chorda tympani mean thickness of the myelin sheath decreased by 14% and 1% respectively (Graphics 11 and 12).

| CAT # | SECTION | UNOPERATED CONTROL (μm) | OPERATED PROXIMAL (μm) | OPERATED DISTAL (μm) |
|---|---|---|---|---|
| 1 | LINGUAL | 1.07 (0.53) | 1.22 (0.59) | |
| | C.T. | 0.56 (0.23) | 0.71 (0.24) | |
| | TOTAL | 0.92 (0.52) | 0.99 (0.53) | 0.71 (0.28) |
| 2 | LINGUAL | 1.17 (0.48) | 1.23 (0.55) | |
| | C.T. | 0.69 (0.24) | 0.58 (0.27) | |
| | TOTAL | 0.96 (0.46) | 1.02 (0.57) | 0.62 (0.22) |
| 3 | LINGUAL | 2.11 (0.83) | 1.03 (0.43) | |
| | C.T. | 0.71 (0.26) | 0.66 (0.21) | |
| | TOTAL | 1.61 (0.22) | 0.98 (0.43) | 0.56 (0.03) |
| 4 | LINGUAL | 1.27 (0.61) | 1.20 (0.68) | |
| | C.T. | 0.77 (0.28) | 0.86 (0.36) | |
| | TOTAL | 1.19 (0.60) | 1.11 (0.63) | 0.63 (0.19) |
| 5 | LINGUAL | 1.16 (1.90) | 1.28 (0.65) | |
| | C.T. | 0.71 (0.23) | 0.72 (0.25) | |
| | TOTAL | 1.04 (1.64) | 1.10 (0.61) | 0.73 (0.27) |
| 6 | LINGUAL | 1.16 (0.52) | 0.89 (0.40) | |
| | C.T. | 0.76 (0.30) | 0.61 (0.22) | |
| | TOTAL | 0.98 (0.48) | 0.82 (0.38) | 0.75 (0.30) |

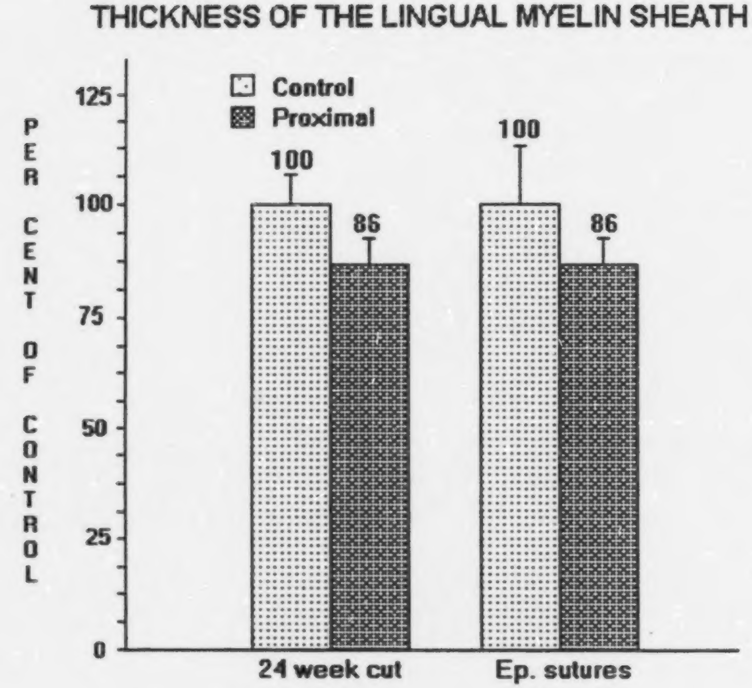
**Table 25:** Thickness of the myelin sheath from lingual, chorda tympani and total values in six cats from group III. Proximal, distal and control sites were analyzed. Obs.: The values are Means and Standard Deviation (S.D.).

| THICKNESS OF THE MYELIN SHEATH FOR GROUP III | P-VALUE |
|---|---|
| CONTROL LINGUAL (µm) X PROXIMAL LINGUAL (µm)<br>1.32 (0.39) 1.14 (0.15) | 0.600 |
| CONTROL C.T. (µm) X PROXIMAL C.T. (µm)<br>0.70 (0.08) 0.69 (0.10) | 0.834 |
| CONTROL TOTAL (µm) X PROXIMAL TOTAL (µm)<br>1.12 (0.26) 1.00 (0.11) | 0.345 |
| CONTROL TOTAL (µm) X DISTAL TOTAL (µm)<br>1.12 (0.26) 0.67 (0.07) | 0.028 |
| PROXIMAL TOTAL (µm) X DISTAL TOTAL (µm)<br>1.00 (0.11) 0.67 (0.07) | 0.028 |

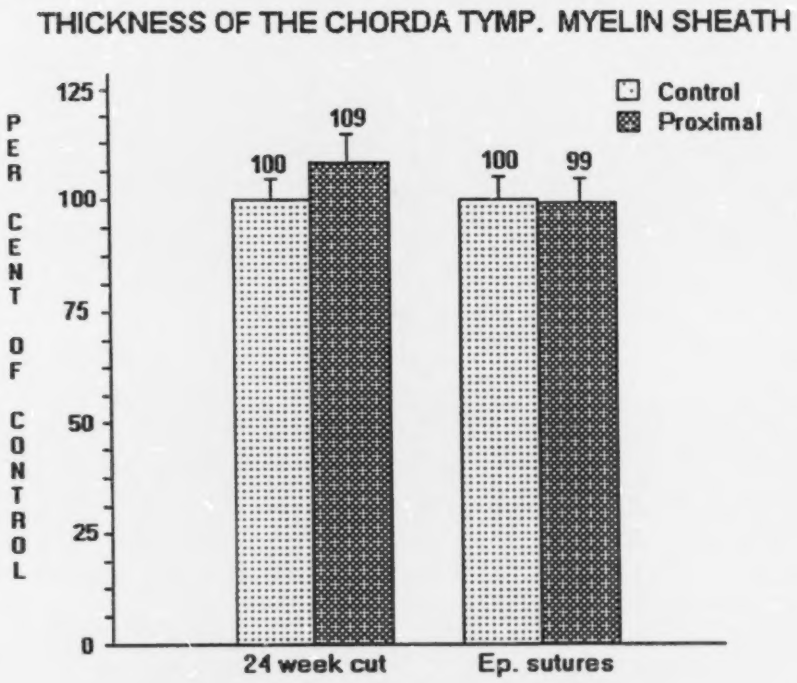
**Table 26:** Comparisons made concerning the thickness of the myelin from group III. Obs.: The values represent means, Standard Deviation (S.D.) and p-value.



**GRAPHIC 10:** Histogram showing the percentage of the total thickness of myelin sheath (lingual and chorda tympani)in control, proximal and distal sections of groups I (12 weeks, unrepaired), II (24 weeks, unrepaired) and III (12 weeks repaired using epineurial sutures). Bar lines show standard errors. The myelin sheaths are reduced distal to the transection. The only difference that was statistically significant($p<0.05$) was between the proximal and control sections in group III (12 weeks, repaired).



**GRAPHIC 11**: Histogram showing the percentage of the lingual myelinated axonal size in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bar lines show standard errors. The lingual nerve is the major contributor for the decrease seen in the total thickness of the myelin sheath proximal to the transection for groups II and III.



**GRAPHIC 12**: Histogram showing the percentage of the chorda tympani myelin sheath thickness in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bar lines show standard errors.

## REMAK BUNDLES

### GROUP I (UNREPAIRED, 12 WEEKS)

There were no statistically significant differences ($p>0.05$) in the total mean number of unmyelinated axons per Schwann cell between the unoperated control nerve (1.85) and the injured nerve at sites either proximal (1.86) or distal (2.14) to the injuries. Nor was there any statistically significant difference ($p>0.05$) between counts taken proximal (1.86) and distal (2.14) to the injury site (Tables 27 and 28).

Proximal and distal to the transection the total mean number of unmyelinated axons per Schwann cell increased by 1% and 15% respectively (Graphic 13).

| CAT # | SECTION | UNOPERATED CONTROL | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| 1 | LINGUAL | 1.95 (1.59) | | |
| | C.T. | 1.70 (1.19) | | |
| | TOTAL | 1.85 (1.38) | 2.36 (1.95) | 2.44 (1.80) |
| 2 | LINGUAL | 2.38 (1.65) | | |
| | C.T. | 1.37 (0.90) | | |
| | TOTAL | 1.98 (1.28) | 1.66 (0.99) | 2.03 (1.77) |
| 3 | LINGUAL | 1.61 (1.10) | | |
| | C.T. | 1.79 (1.80) | | |
| | TOTAL | 1.72 (1.45) | 1.56 (1.00) | 1.95 (1.39) |

**Table 27:** Mean number of unmyelinated axons per Schwann cell from lingual, chorda tympani and total values from group I. Proximal, distal and control sites were analyzed. Obs.: The values are means and Standard Deviation (S.D.).

| REMAK BUNDLES FOR GROUP I | | | P-VALUE |
|---|---|---|---|
| CONTROL TOTAL<br>1.85 (0.13) | X | PROXIMAL TOTAL<br>1.86 (0.44) | 1.000 |
| CONTROL TOTAL<br>1.85 (0.13) | X | DISTAL TOTAL<br>2.14 (0.26) | 0.109 |
| PROXIMAL TOTAL<br>1.86 (0.44) | X | DISTAL TOTAL<br>2.14 (0.26) | 0.109 |

**Table 28:** Comparisons made concerning the number of unmyelinated axons per Schwann cell from group I. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.

**GROUP II (UNREPAIRED, 24 WEEKS)**

There were no statistically significant differences ($p>0.05$) in the total mean number of unmyelinated axons per Schwann cell between unoperated control nerve (2.31) and the injured nerve at sites either proximal (2.21) or distal (2.04) to the injuries. Nor was there any statistically significant differences ($p>0.05$) between counts taken proximal (2.21) and distal (2.04) to the injury site (Tables 29 and 30).

There were no significant differences ($p>0.05$) for the lingual and chorda tympani number of unmyelinated axons per Schwann cell between proximal and control sections (Tables 29 and 30). The mean values for the lingual nerve in the control and proximal sections were 2.43 and 2.28 respectively. Similarly, the mean values for the chorda tympani nerve were 2.16 and 2.03 respectively.

Proximal and distal to the transection the total mean of unmyelinated axons per Schwann cell decreased by 4% and 12% respectively (Graphic 13). Proximal to the transection the lingual and chorda tympani mean of unmyelinated axons per Schwann cell decreased by 6% (Graphics 14 and 15).

| CAT # | SECTION | UNOPERATED CONTROL | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| 1 | LINGUAL | 2.16 (1.64) | 1.76 (1.06) | 2.32 (1.93) |
| | C.T. | 2.82 (2.63) | 1.64 (1.22) | 1.68 (1.05) |
| | TOTAL | 2.22 (1.72) | 1.72 (1.12) | 2.11 (1.75) |
| 2 | LINGUAL | 2.18 (1.49) | 2.25 (1.76) | |
| | C.T. | 2.39 (2.10) | 1.85 (1.26) | |
| | TOTAL | 2.27 (1.77) | 2.16 (1.67) | 1.93 (1.34) |
| 3 | LINGUAL | 2.59 (1.99) | 1.70 (1.16) | |
| | C.T. | 1.62 (1.07) | 1.60 (0.97) | |
| | TOTAL | 2.29 (1.80) | 1.67 (1.09) | 1.61 (1.05) |
| 4 | LINGUAL | 3.14 (2.25) | 2.88 (1.97) | |
| | C.T. | 1.72 (1.33) | 2.74 (1.86) | |
| | TOTAL | 2.61 (2.12) | 2.86 (2.01) | 2.24 (1.64) |
| 5 | LINGUAL | 2.09 (1.70) | 2.80 (2.30) | |
| | C.T. | 2.23 (1.96) | 2.32 (1.50) | |
| | TOTAL | 2.17 (1.81) | 2.65 (2.07) | 2.30 (1.89) |

**Table 29:** Mean number of unmyelinated axons per Schwann cell for lingual, chorda tympani and total values from group II. Proximal, distal and control sites were analyzed. Obs.: The values are Means and Standard Deviation (S.D.).

| REMAK BUNDLES FOR GROUP II | | | P-VALUE |
|---|---|---|---|
| CONTROL LINGUAL<br>2.43 (0.44) | X | PROXIMAL LINGUAL<br>2.28 (0.56) | 0.500 |
| CONTROL C. TYMPANI<br>2.16 (0.49) | X | PROXIMAL C. TYMPANI<br>2.03 (0.49) | 0.686 |
| CONTROL TOTAL<br>2.31 (0.17) | X | PROXIMAL TOTAL<br>2.21 (0.54) | 0.500 |
| CONTROL TOTAL<br>2.31 (0.17) | X | DISTAL TOTAL<br>2.04 (0.28) | 0.138 |
| PROXIMAL TOTAL<br>2.21 (0.54) | X | DISTAL TOTAL<br>2.04 (0.28) | 0.345 |

**Table 30:** Comparisons made concerning the number unmyelinated axons per Schwann cell from group II. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.

**GROUP III (REPAIRED, 12 WEEKS)**

There were no statistically significant differences ($p>0.05$) in the total mean number of unmyelinated axons per Schwann cell between the unoperated control nerve (2.48) and the injured nerve proximal (2.46) to the injury. Nor was there any statistically significant differences ($p>0.05$) between the counts taken proximal (2.46) and distally (2.09) to the injury site. Distal to the site of injury, total counts of unmyelinated axons per Schwann cell were reduced (2.09) and the difference was statistically significant from the control (2.48) ($p<0.05$) (Tables 31 and 32).

There were no significant differences ($p>0.05$) for the lingual and chorda tympani number of unmyelinated axons per Schwann cell between proximal and control sections (Tables 31 and 32). The mean values for the lingual nerve in the control and proximal sections were 2.67 and 2.65 respectively. Similarly, the mean values for the chorda tympani nerve were 2.08 and 2.03.

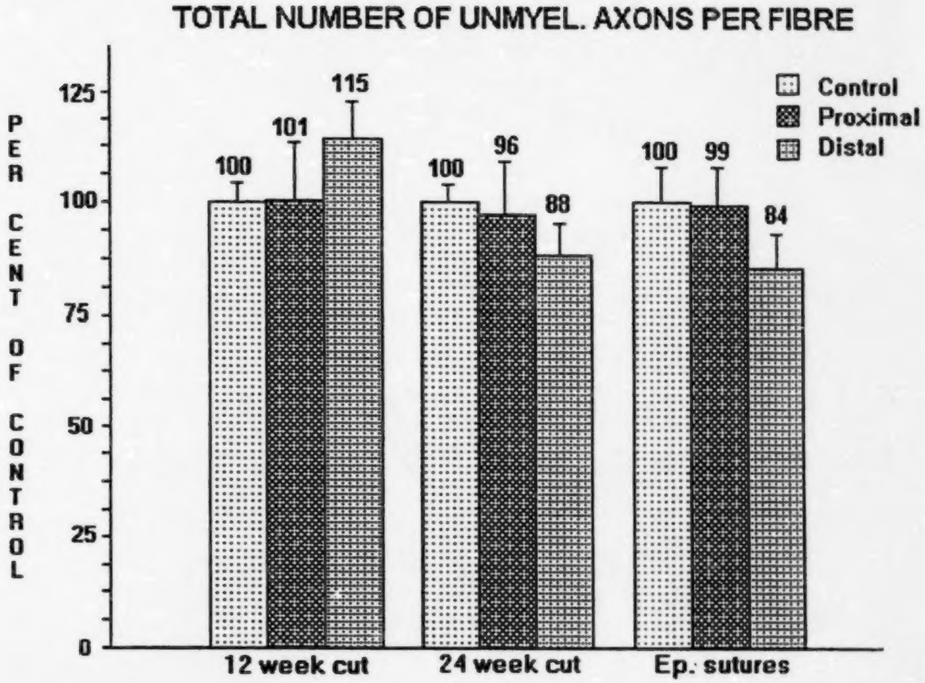
Proximal and distal to the injury, the total number of unmyelinated axons per Schwann cell decreased by 1% and 16% respectively (Graphic 13). Proximal to the injury the lingual and chorda tympani number of unmyelinated axons per Schwann decreased by 1% and 2% respectively (Graphics 14 and 15).

| CAT # | SECTION | UNOPERATED CONTROL | OPERATED PROXIMAL | OPERATED DISTAL |
|---|---|---|---|---|
| 1 | LINGUAL | 3.19 (2.51) | 3.38 (2.39) | |
| | C.T. | 1.81 (1.53) | 2.29 (1.80) | |
| | TOTAL | 2.69 (2.31) | 3.03 (2.29) | 2.60 (1.97) |
| 2 | LINGUAL | 2.31 (1.51) | 3.02 (2.09) | |
| | C.T. | 1.88 (1.15) | 2.39 (2.07) | |
| | TOTAL | 2.08 (1.36) | 2.82 (2.10) | 1.58 (1.38) |
| 3 | LINGUAL | 3.51 (2.91) | 3.12 (2.98) | |
| | C.T. | 2.42 (2.13) | 1.95 (1.43) | |
| | TOTAL | 2.99 (2.64) | 2.90 (2.79) | 2.29 (1.95) |
| 4 | LINGUAL | 2.22 (1.89) | 1.92 (1.51) | |
| | C.T. | 1.78 (1.37) | 1.62 (1.18) | |
| | TOTAL | 2.14 (1.80) | 1.85 (1.45) | 1.72 (1.10) |
| 5 | LINGUAL | 2.01 (1.60) | 2.21 (1.69) | |
| | C.T. | 2.22 (1.76) | 2.09 (1.82) | |
| | TOTAL | 2.09 (1.66) | 2.16 (1.75) | 2.17 (1.52) |
| 6 | LINGUAL | 2.80 (2.30) | 2.27 (1.93) | |
| | C.T. | 2.37 (2.08) | 1.83 (1.37) | |
| | TOTAL | 2.68 (2.25) | 2.14 (1.80) | 2.19 (1.76) |

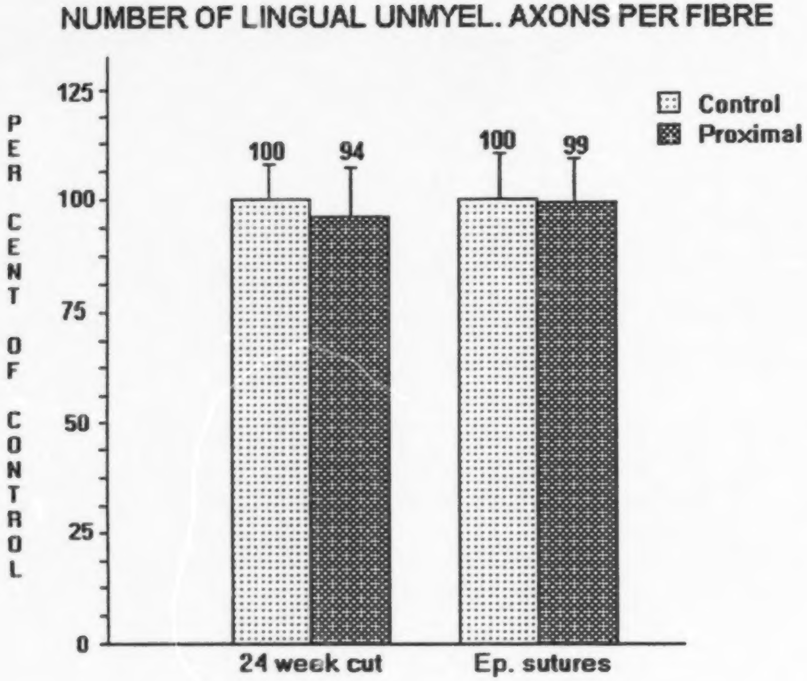
**Table 31:** Mean number of unmyelinated axon per Schwann cell from group III. Proximal, distal and control sites were analyzed. Obs.: The values are Means and Standard Deviation.

| REMAK BUNDLES FOR GROUP III | | | P-VALUE |
|---|---|---|---|
| CONTROL LINGUAL | X | PROXIMAL LINGUAL | |
| 2.67 (0.59) | | 2.65 (0.59) | 0.753 |
| CONTROL C. TYMPANI | X | PROXIMAL C. TYMPANI | |
| 2.08 (0.29) | | 2.03 (0.29) | 0.753 |
| CONTROL TOTAL | X | PROXIMAL TOTAL | |
| 2.48 (0.40) | | 2.46 (0.51) | 0.753 |
| CONTROL TOTAL | X | DISTAL TOTAL | |
| 2.48 (0.40) | | 2.09 (0.38) | 0.046 |
| PROXIMAL TOTAL | X | DISTAL TOTAL | |
| 2.46 (0.51) | | 2.09 (0.38) | 0.116 |

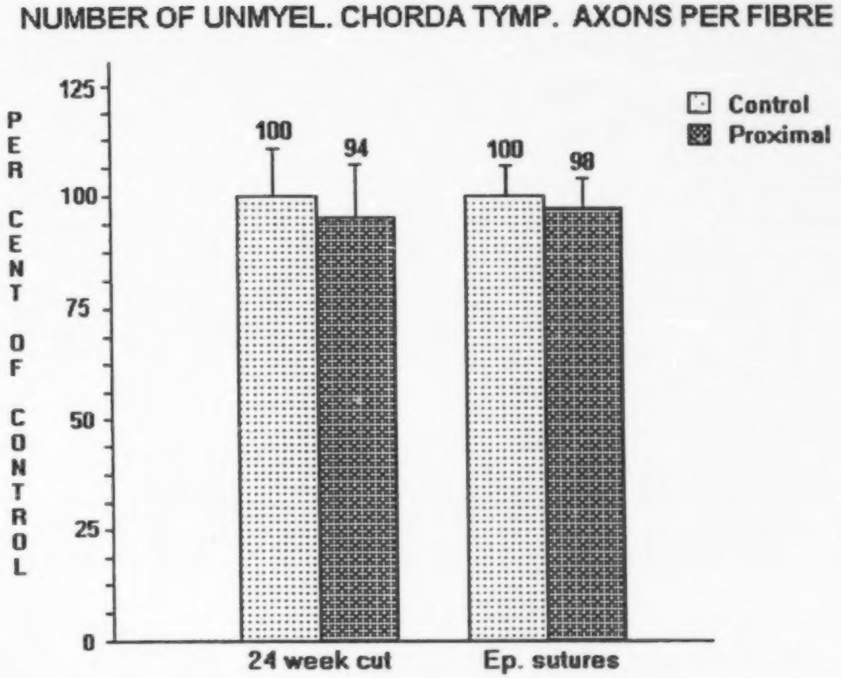
**Table 32:** Comparisons made concerning the number of unmyelinated axons per Schwann cell. Obs.: The values represent Means, Standard Deviation (S.D.) and p-value.



**GRAPHIC 13:** Histogram showing the percentage of the total (lingual and chorda tympani) number of unmyelinated axons per Schwann cell in control, proximal and distal sections of groups I (12 weeks, unrepaired), II (24 weeks, unrepaired) and III (12 weeks repaired using epineurial sutures). Bar lines show standard errors. The only difference that was statistically significant ($p<0.05$) was between distal and control values in group III.



**GRAPHIC 14**: Histogram showing the percentage of the lingual unmyelinated axons per Schwann cell in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bar lines show standard errors.



**GRAPHIC 15**: Histogram showing the percentage of the chorda tympani unmyelinated axons per Schwann cell in control and proximal sections of groups II (unrepaired, 24 weeks) and III (repaired, 12 weeks). Bar lines show standard errors.

# CHAPTER FIVE

# DISCUSSION

## INTRODUCTION

Proximal to the injury it was not possible to identify the lingual and the chorda tympani nerves as independent entities in most of the specimens from group I. This is probably because, after transection, the nerves in this group had only 12 weeks to regenerate and this may not be long enough for the smaller axons of the chorda tympani nerve to recover from the trauma and reorganize as an independent bundle. The only specimen in which was possible to identify the chorda tympani nerve, presented some degenerating axons and revealed fewer myelinated axons (Table 3).

This was not true for group II (unrepaired, 24 weeks) or group III (repaired, 12 weeks). This suggests that immediate suturing accelerated lingual nerve recovery.

Distal to the injury, it was not possible to distinguish the lingual from the chorda tympani for all the animals in groups I and III and most animals of group II. Interestingly, group II had one animal in which the chorda tympani and the lingual nerves could be seen as independent entities (cat one of group II). This shows a good recovery of this particular animal. However, the fact that only one animal out of five showed these characteristics does not suggest that mismatching is less likely to occur in unrepaired nerves.

## NUMBER OF MYELINATED AXONS PROXIMAL TO THE INJURY

Others have reported no significant difference between the number of myelinated axons proximal to an injury and control nerves (Horch and Lisney, 1981; Carter and Lisney, 1987, Peyronnard et al., 1988; Holland and Robinson, 1990-a). This is in agreement with present studies whether the nerve is repaired or not.

## TOTAL MYELINATED COUNTS

There were no significant difference in the total number of myelinated axons proximal to the injury in all the three groups. Tables 3 and 4 show that there was a decrease of 22% and 9% for groups I and II respectively. For group II, it might be due to either a loss of nerve cells after the injury, or to dying back of a few millimetres of some axons. It is also possible that both events happened, or that cell death occurred to a larger extent and that sprouts are attenuating the loss. However, others have reported no evidence of myelinated neuron death after damage (Carter and Lisney, 1987).

A greater reduction of 22% of myelinated axon in group I can be explained by the hypotheses listed above for group II. It is also related to the absence of the chorda tympani in the proximal sections of group I. This may be the result of a more intense retrograde reaction suffered by the smaller chorda tympani axons. In the only specimen, in which it was possible to recognize the chorda tympani nerve, there was some degree of axonal degeneration and only a few chorda tympani axons were present. That, for group II, the chorda tympani could be readily identified as a distinct trunk supports these suggestions.

It was possible to identify the lingual and the chorda tympani nerves as independent nerve trunks proximal to the injury site in group III. This suggests that epineurial suturing had a positive effect in accelerating regeneration. Group III had a 14% increase in the number of myelinated axons. This indicates good regeneration with no axonal loss. This increase may be attributed to sprouting proximal to the injury. Risling et al. (1983) demonstrated that sprouting can occur proximal to the injury, although Horch and Lisney (1981) support the idea that sprouting occurs at or beyond the level of the neuroma.

The fate of the cell bodies is not known. An increase in the number of myelinated axons proximal to the injury was observed but this does not exclude the possibility of some extent of cell death. Sprouting of the surviving axons may mask any cell loss.

## LINGUAL AND CHORDA TYMPANI COUNTS

The number of lingual and chorda tympani myelinated axons proximal to the injury was not significantly different from the control in groups II and III. For group II, it decreased by 8% in the lingual and 11% in the chorda tympani, a similar percentage to the value found for the total counts. A more prominent decrease in the number of myelinated axons in the chorda tympani nerve suggested that these smaller axons do not react to injury in the same way that lingual axons do.

For group III, the number of myelinated axons proximal to the injury increased by 27% in the lingual nerve and decreased by 12% in the chorda tympani nerve. The fact that a substantial increase in the lingual axonal population was found can be related to either more vigorous sprouting or to a shorter recovery time. The shorter recovery time might have affected the number of myelinated axons proximal to the injury. This is due to formation of sprouts proximal to the injury that would probably have gone further down if the animals had survived longer. Similarly, the fact that the number of chorda tympani axons decreased can be attributed to the shorter recovery time. It thus appears that this population of smaller fibres is more seriously affected by the injury and needs a longer time for regeneration. Comparing group I with group III it seems that the sutures did help to accelerate this process. In only one specimen in group I the chorda tympani could be identified proximal to the injury revealing a markedly low value (Table 3, cat # 2). This suggests that 12 weeks after transection the chorda tympani axons are not ready to cross the injury. The mechanism by which myelinated axons differ in responding to trauma is not clear (Risling et al., 1983).

## NUMBER OF MYELINATED AXONS DISTAL TO THE INJURY

Distally to the injury it was not possible to analyze the lingual and the chorda tympani separately. This indicates that none of the groups offered an adaptation that prevented misdirection from occurring. Thus, the randomness of growth did not allow the lingual and the chorda tympani to remain segregated. This finding helps a better understanding of the lack of regeneration of gustatory fibres as opposed to the sensory fibres (Girod et al., 1989). It also supports the suggestion, made by Robinson and Winkles (1991), of reduced taste bud reinnervation by the chorda tympani after lingual nerve injury. This assumption was because fewer taste buds were present on fungiform papillae of the anterior two-thirds of the tongue after lingual nerve transection (Robinson and Winkles, 1991). The results reported here are in agreement with Robinson's (1989) experiment. In his study significantly fewer gustatory and thermosensitive stimuli could be recorded in the chorda tympani nerve after transection (Robinson, 1989).

Group II had a significant reduction of the total number of myelinated axons distally to the injury. Groups I and III did not show any significant difference although a decrease of 13% and 7% was detected. This is probably because the animals from group II had a longer survival time than those of groups

I and III. This suggests that the less prominent decrease seen in groups I and III is due to sprouts that are well represented distally 12 weeks after transection. Another possibility could be that after suturing more myelinated axons crossed the injury site. This suggestion is due to the fact group III had a reduction in the number of myelinated axons of only 7% as opposed to 13% in group I. Moreover, proximal to the injury the chorda tympani nerve could not be identified in group I. That the only specimen in which it was possible to identify the chorda tympani as a distinct trunk contained fewer myelinated axons supports this idea. Therefore, it seems that in group I either very few or none of the chorda tympani axons managed to cross the injury site.

Jenq and Coggeshall (1985-a) found a significant increase in the number of myelinated axons distal to the injury. This was true after crushing, transecting and removing a 4-mm nerve segment of the rat sciatic nerve. Initially the differences between the results reported here and their experiment could be explained by the even shorter time recovery of only eight weeks in their experiment. However, further experiments (Jenq and Coggeshall, 1985-b) still reveal a marked increase following 4-mm nerve segment and survival time of nine months. On the other hand, the fact that the nerve stumps were placed in a silicone tube, resulting in a very good morphological appearance might be another possible explanation.

Other experiments (Carter and Lisney, 1987; Pover and Lisney, 1988) support the idea that there is an increase in the number of myelinated axons distal to the injury. This is probably due to one regenerated myelinated axon being able to support more than one sprout in the distal stump. These contradictory results may result from the lingual and chorda-tympani myelinated axons that could not continue to cross the injury site and ended blindly in the neuroma. Another explanation is that part of the unmyelinated axons found distally to the injury would be sprouts of myelinated axons. The latter suggestion would overestimate the population of unmyelinated axons and underestimate that of myelinated axons. The fact that the animals of Pover and Lisney's (1988) had a long recovery time supports the latter idea.

## TOTAL NUMBER OF UNMYELINATED AXONS PROXIMAL TO THE INJURY

### TOTAL COUNTS

The number of unmyelinated axons proximal to the injury site did not differ significantly from the control in any of the groups. However, there was an increase of 10%, 46% and 47% for groups II, III and I respectively. This can be explained by a higher incidence of sprouts in the groups that had a 12-week recovery time (groups I and III). On the other hand, group II still showed a 10% increase. This might be due to the presence of sprouts that are still present 24 weeks after injury. This may further decrease if a longer survival period was allowed for regeneration. Another explanation could be a lack of success of some axons in crossing the injury site. These axons would find their way out growing back proximally as recurrent axons (Aldskogius and Risling, 1983). Central to transection injuries, the number of unmyelinated axons either does not alter (Holland and Robinson, 1990-a) or decreases (Carter and Lisney, 1987). Therefore, it is most likely that sprouts contributed to the 10% increase in the number of unmyelinated axons proximal to the transection. This value would probably have been reduced further if the animals had survived longer.

## LINGUAL AND CHORDA TYMPANI COUNTS

There was no significant difference in the number of lingual or chorda tympani unmyelinated axons proximal to the injury for either of the groups. The lingual nerve values increased by 24% and 59%, and the chorda tympani by 3% and 16% for groups II and III respectively. The axonal counts were more elevated in group III than in group II. This is explained by the same factors that were mentioned above to explain the results found for the total counts. The lingual nerve is the major contributor to that increase. This is supported by the more evident chromatolysis reaction seen in small neurons in the dorsal root ganglia of L7 in cats (Risling et al., 1983). There is also the possibility that some post-ganglionic unmyelinated axons that re-enter the lingual-chorda nerve were transected. The damage site would be close to their cell bodies within the submandibular ganglion and thus likely to lead to neuronal death.

## NUMBER OF UNMYELINATED AXONS DISTAL TO THE INJURY

For group II, there was not a significant difference in the number of unmyelinated axons distal to the injury. However, there was a significant increase for group III. Group I had an enormous increase, but the values did not show any statistically significant difference presumably due to the small number of animals analyzed in this group.

The small decrease seen in group II is in agreement with that found distal to the injury (Jenq and Coggeshall, 1984; Lisney, 1987). Lisney (1987) claims that most of the unmyelinated axons that survive the injury can regenerate into the distal stump. This would be the same proportion found for myelinated axons. He used electrodes distal to saphenous transection after various periods of survival time to estimate the proportion of unmyelinated axons that can cross the site of injury. By eight weeks after injury, a large proportion of unmyelinated axons could be activated when stimulus were applied distal to the injury. Results from one animal showed that 91% of the unmyelinated axons tested could be activated from the distal stump after 26 weeks. This does not assure that these axons are not sprouts of myelinated axons. It is not possible to say that the number of unmyelinated axons found distally to the injury is the proportion of unmyelinated axons that managed to cross the injury.

There was a statistically significant increase in the number of unmyelinated axons for group III. Group I also had an enormous increase. This is because of the shorter survival time that the animals of these groups had. It also shows that 3 months after injury many sprouts are still represented distal to the injury site. The findings by Fried and Erdélyi (1982) that four months after transection the number of unmyelinated axons were higher than the control returning to normal by 7 months supports the above hypothesis.

### MYELINATED AXONAL SIZE PROXIMAL TO THE INJURY

There is a decrease in size of myelinated fibres proximally to injury (Gutmann and Sanders, 1943; Weiss et al., 1945; Gutmann and Holubar, 1951; Aitken and Thomas, 1962; Holland and Robinson, 1990-a). Some studies found that the myelinated fibres did not recover their original size even after 300 days after damage (Gutmann and Sanders, 1943; Aitken and Thomas, 1962, Gutmann and Holubar, 1951). Transection and transection followed by suturing revealed a 10.2% reduction of the mean size of myelinated axons between 50 and 150 days following injury. After 225 days the axon diameter presented values close to normal (Cragg and Thomas, 1961).

In the present study the size of the myelinated axons proximal to the injury did not differ from the control. The values were larger proximal to the injury for groups II and III. The time recovery of these animals is shorter than those of Gutmann and Sander (1943) or Aitken and Thomas (1962). Therefore, it is unlikely that maturation of the myelinated fibres occurred earlier. Group I showed even larger fibres. This is probably due to absence of the chorda tympani axons in two animals of this group. The axons were not measured in the only identifiable chorda tympani nerve.

These findings are on agreement with experiments that revealed a better recovery of the larger mechanosensitive fibres over the smaller thermosensitive and gustatory fibres (Robinson, 1989). However, the secretomotor and vasomotor fibres, which are also represented by small myelinated and unmyelinated fibres, had better recovery than the thermosensitive and gustatory fibres (Robinson, 1989).

Although different axonal populations respond differently to nerve injury the reason for this is not clear. For instance, following sciatic injury there was a better recovery of the motor over the sensory myelinated axons in the roots of L7 (Risling et al., 1983). Following the same kind of injury, one of its tributaries had a significantly higher number of unmyelinated axons than the other (Jeng and Coggeshall, 1985-b). In group II, the chorda tympani nerve had a more prominent increase in size of myelinated fibres proximal to the injury than the lingual nerve. In group I, it was not possible to identify the lingual and the chorda tympani separately. This suggests that the smaller myelinated fibres of the chorda tympani nerve were more severely affected by the injury than those of the lingual nerve.

## MYELINATED AXONAL SIZE DISTAL TO THE INJURY

The literature shows that after transection there is a myelinated axonal size reduction distal to the injury that remains even in late stages of recovery (Fried and Erdélyi, 1982; Horch and Lisney, 1981).

In the present study this was true for all the groups but only statistically significant for group III probably due to the shorter recovery time. There is evidence that time allows an increase in the myelinated axonal size following injury (Gutmann and Sanders, 1943; Cragg and Thomas, 1961). Group I, which also had a shorter time recovery of three months, had a substantial mean difference between control and distal section (Table 9). However, these values did not show a statistically significant difference probably because of a few animals present in this group. Group II, which had a longer survival time, probably had the chance to further increase the size of its axons. Thus, a smaller difference between the mean size of the myelinated axons was observed.

Changes in fibre size proximal to the injury are strongly related to altered conduction velocities following injury (Cragg and Thomas, 1961). The increase in myelinated axonal size proximal to the injury does not support the findings of decreased conduction velocities of the lingual nerve observed by Robinson (1989, 1992). This increase reflects the absence of smaller axons in these sections. The myelinated axonal size distal to the injury is in agreement with Robinson's findings.

## THICKNESS OF THE MYELIN SHEATH PROXIMAL TO THE INJURY

The thickness of the myelin sheath increased slightly following various kind of nerve injury between 7 and 50 days (Cragg and Thomas, 1961). However, it decreased between 50 and 150 days leading to values close to normal levels (Cragg and Thomas, 1961).

In regenerated nerves reduced conduction velocities are related to thin myelin thickness (Sanders and Whitteridge, 1946). However, Cragg and Thomas (1961) showed that the axon diameter is more strongly related to conduction velocities. Cragg and Thomas' conclusions were based on the fact that slow conducting axons were smaller axons but had thicker myelin sheaths than controls. They suggested that thicker myelin sheaths resulted from absorbency of water rather than production of more myelin.

Their suggestion was probably true because it is known that both the myelin sheath and the axon size contribute to axonal conduction velocity. A thinner myelin sheath would increase the leakage of current across the myelin. A smaller axon would offer an increased resistance to the flow of ions within the axon. Therefore, small axons with thinner myelin sheath have lower conduction velocities. The thickness of the myelin sheath proximal to the injury does not differ from the control following nerve transection. When axons are prevented from regenerating, a neuroma with smaller axons and a thinner myelin sheath is formed (Holland and Robinson, 1990-a).

After nerve crush the conduction velocities of the myelinated axons were close to normal in both the proximal and distal nerve segments. Eighteen months after transection the conduction velocities proximal to the injury were also close to normal. The values in the distal stump were about 50% of its control values (Horch and Lisney, 1981).

In the study reported here there was no significant difference in the thickness of the myelin proximal to the injury for any of the groups. This was true for total values in all groups, and for lingual and chorda tympani values in groups II and III. There was a slight decrease in the thickness of the myelin sheath for total values in groups II and III and an increase for group I. This can be attributed to the absence of chorda tympani axons in these sections. The results reported in this section are in agreement with recent observations (Holland and Robinson, 1990-a).

## THICKNESS OF THE MYELIN DISTAL TO THE INJURY

There was a decrease in the total myelin thickness of 22%, 41%, and 27% for groups II, III, and I respectively. It was statistically significant only for group III. This may be explained by the different survival time that each group had. Groups I and III, which had three months recovery time, had a more prominent reduction in the myelin sheath than group II. The smaller reduction in the myelin thickness of group I when compared with group III seems difficult to explain. However, the fact that in group I the chorda tympani axons were not well represented in proximal sections suggests that its absence in distal sections masked the results. This is in conflict with Holland and Robinson (1990-a). They did not report any significant change of the myelin thickness distal to the injury, yet their animals also had about three months to recover from the injury. The contradiction between the results reported here and that of Holland and Robinson's (1990-a) is probably due to better response to injury of the inferior alveolar nerve.

## REMAK BUNDLES PROXIMAL AND DISTAL TO THE INJURY

There was no significant difference in the number of unmyelinated axons per Schwann cell between control and proximal sections, for any of the groups. The results seem very similar for the lingual, chorda tympani and total nerve values. This is in contradiction to some authors who found a significant decrease after 6 months following transection (Carter and Lisney, 1981). Holland and Robinson (1990-a) also reported a substantial decrease in the size of the Schwann cell unit three months following inferior alveolar nerve transection. On the other hand, Fried and Erdélyi (1982) show that between two and four months after a 4 or 8mm nerve-segment removal there was an increase in the size of the Schwann cell unit. The very similar values found in control and proximal sections in the study reported here is probably due to an increase in the Schwann cell unit in the first stages of nerve regeneration. Although Carter and Lisney (1981) found a decrease 6 months after injury, the values proximal to the injury were slightly lower than that of the control. Three months after injury they were higher. These facts reinforce the above suggestion.

Distal to the injury, the literature also provides contradictory results. Holland and Robinson (1990-a) did not find any change in the Schwann cell unit following transection. Fried and Erdélyi (1982) found that it appeared smaller at 2, 4 and 11 months following neurectomy. Eleven months after injury the decrease was more prominent.

In this study, group I had an increase of 15.68% in the Schwann cell size unit. Group II had a decrease of 11.69% and group III had a statistically significant smaller Schwann cell units ($p<0.05$). If Fried and Erdélyi's (1982) findings can be applied here, it can be assumed that group III, which is the repaired group, had faster recovery than group I. However, it leaves unanswered the fact that no statistic significant difference was found for group II.

# CHAPTER SIX

# CONCLUSIONS

## INTRODUCTION

Lingual nerve injury has been reported to occur frequently following lower third molar extractions (Mason, 1988; Blackburn and Brammley, 1989). If transection occurs, the cut ends tend to be pulled apart compromising the extent of recovery. Robinson (1989) demonstrated through electrophysiological studies that, after transection, lingual nerve activity was significantly reduced. As epineurial sutures are being employed as a method of repair following nerve injuries (Riediger et al., 1989), this study was aimed at estimating its effectiveness in allowing good recovery of the lingual and chorda tympani axons.

Fourteen cats were assigned to three groups, which consisted: I) transected with 3 months recovery time. II) transected with 6 months recovery time. III) transected and immediately repaired with 3 months recovery time. The number and size of myelinated axons as well as the thickness of their myelin sheath were determined central and distal to transection site and from the control side. The number of unmyelinated axons per Schwann cell was also counted at the same corresponding sites.

The present results demonstrated an increase of 14% in the number of myelinated axons proximal to the transection in group that was repaired. Distal to the transection, the group that had longer recovery time had a significant decrease ($p<0.05$) in the number of myelinated axons, but presented larger myelinated axons than the others. The number of unmyelinated axons distal to the transection were increased in the groups that had shorter recovery time.

## SUMMARY OF THE RESULTS FOUND

1 Although the results did not show any differences that were statistically significant in the total number of myelinated axons proximal to the transection, there was an increase for the group that was repaired and a decrease for the unrepaired groups. The increase observed in the repaired group was restricted only to the lingual nerve. The chorda tympani counts decreased by 13.5%.

2 Distal to the injury, there was a statistically significant decrease in the number of myelinated axons for the group that had the longer recovery time. The others had a less prominent decrease that was not statistically significant.

3 Although not significant, there was an increase of approximately 45% in the number of unmyelinated axons proximal to the injury for the groups that had a shorter recovery time. The group with longer survival time had 10% increase. The lingual nerve again was the major contributor to this increase.

4 There was a statistically significant ($p<0.05$) increase in the number of unmyelinated axons distal to the transection for the repaired group. The group with shorter recovery time and no repair had a 352% increase. The group with longer recovery time had a 10% increase.

5 Although not significant, there was an increase in the myelinated axonal size proximal to the injury of 22% in the

group that was not repaired and had shorter recovery time. The chorda tympani nerve seems to be the major contributor for this increase.

6 Distal to the injury, there was a decrease of 28% and 39% in the myelinated axonal size for the groups with shorter recovery time. This difference was statistically significant ($p<0.05$) only in the repaired group. The group with longer survival time had a 10% decrease in size.

7 Proximal to the injury, there was an increase in the thickness of the myelin sheath of 29% for the group that was not repaired and had shorter survival time. The group that had a longer survival time and the repaired group had a decrease of 7% and 11% respectively.

8 Distal to the injury, there was a statistically significant ($p<0.05$) decrease in the thickness of the myelin sheath for the group that was repaired. The unrepaired groups had a decrease of about 24%.

9 Proximal to the transection the number of unmyelinated axons per Schwann cell did not differ from the control.

10 Distal to the transection, the group that was not repaired and had shorter recovery time had an increase of 15%. The group that had longer survival time had a decrease of 16%. The repaired group had a statistically significant decrease ($p<0.05$).

## CONCLUSIONS

The following conclusions may be stated within the limitations of this study:

1 The data regarding the number of myelinated proximal to the injury suggests that immediate epineurial suturing is an effective method of lingual nerve repair.

2 The fact that after transection following no repair, the lingual and the chorda tympani could not be identified proximal to the injury as independent nerve trunks indicates that immediate epineurial suturing accelerated the process of regeneration.

3 The results following immediate epineurial suturing and 12 weeks survival time were very similar to that of no repair and 24 weeks survival time.

4 The fact that distal to the transection it was not possible to identify the lingual and the chorda tympani as independent nerve trunks shows that immediate epineurial suturing did not prevent misdirection.

5 Future studies assessing morphological characteristics of lingual nerve recovery should be designed considering a

longer survival time.

6 The differences between the groups that were not repaired are due to a more advanced stage of maturation of the group that had a longer survival time. Distal to the transection, the group that had a longer survival time had larger myelinated axons with thicker myelin sheath than the group that had a shorter recovery time.

# REFERENCES

Aitken JT, and Thomas PK. (1962). Retrograde changes in fibre size following section. Journal of Anatomy. Vol:96 (121-129).

Aldskogius H, and Risling M. (1983). Preferential loss of unmyelinated L7 dorsal root axons following sciatic nerve resection in kittens. Brain Research. Vol: 289 (358-361).

Biedenbach MA, Beuerman RW, and Brown AC. (1975). Graphic-digitizer analysis of axon spectra in ethmoidal and lingual branches of the trigeminal nerve. Cell and Tissue Research. Vol:157 (341-352).

Blackburn CW, and Brammley PA. (1989). Lingual nerve damage associated with the removal of lower third molars. British Dental Journal. Vol: 167 (103-107).

Blackburn CW. (1990). A method of assessment in cases of lingual nerve injury. British Journal of Oral Maxillofacial Surgery. Vol: 28 (238-245).

Blake P. (1989). Excision of the submandibular gland: minimizing the risk of nerve damage: comment. Australian New Zealand Surgery Vol: 59 (284).

Bruce RA, Frederickson GC, and Small GS. (1980). Age of patients and morbidity associated with mandibular third molar surgery. Journal of the American Dental Association. Vol: 101 (240-245).

Carter DA, and Lisney SJW. (1987). The numbers of unmyelinated and myelinated axons in normal and regenerated rat saphenous nerves. Journal of the Neurological Sciences. Vol: 80 (163-171)

Cogswell WW. (1942) Surgical problems involving the mandibular nerve. Journal of American Dental Association. Vol:29 (964-967).

Cowan PW. (1990). 'Atrophy of fungiform papillae following lingual nerve damage'- A suggested mechanism [letter]. British Dental Journal. Vol: 168 (95).

Cragg BG, and Thomas PK. (1961). Changes in conduction velocity and fibre size proximal to peripheral nerve lesions. Journal of Physiology. Vol: 157 (315-327).

Davis WH, Hochwald DA, and Kaminishi RM. (1983). Modified distolingual splitting technique for removal of impacted mandibular third molars: technique. Oral Surgery. Vol: 56 (2-8).

Donoff RB, and Guralnick W. (1982). The application of microneurosurgery to oral-neurologic problems. American Association of Oral and Maxillofacial Surgeons. Vol: 300 (156-159).

Dubner R, Sessle BJ, and Storey AT. (1978). The neural basis of oral and facial function. Plenum Press. New York and London.

Edds MV. (1945). Prevention of nerve regeneration and neuroma formation by caps of synthetic resin. Journal of Neuroscience. Vol: 2 (507-509).

Erlanger J, and Gasser H. (1937). Electrical signs of nervous activity. University of Pennsylvania Press, Philadelphia.

Foley JO. (1945). The sensory and motor axons of the chorda tympani. Proceedings Society of Experimental Biology and Medicine. Vol: 60 (262-267).

Freed, Medinaceli L, and Wyatt RJ. (1985). Promoting functional plasticity in the damaged nervous system. Science. Vol: 227 (1544-1552).

Fried K and Erdélyi G. (1982). Inferior alveolar nerve regeneration and incisor pulpal reinnervation following intramandibular neurotomy in the cat. Brain Research. Vol: 244 (259-268).

Gelberman RH. (1991). Operative nerve repair and reconstruction. Vol: 1. J. B. Lippincott Company. Phyladelphia.

Gibby WA, Koerber HR, and Horch KW. (1983). A quantitative evaluation of suture and tubulization nerve repair techniques. Journal of Neurosurgery. Vol: 58 (574-579).

Girod SC, Neukkam FW, Girod B, Reumann K, and Semrau H. (1989). The fascicular structure of the lingual nerve and the chorda tympani: an anatomical study. Journal of Oral Maxillofacial Surgery. Vol: 47 (607-609).

Gutmann E, Guttmann L, and Medawar PB. (1942-a). The rate of regeneration of nerve. Journal of Experimental Biology. Vol: 1 (14-44).

Gutmann E, and Sanders FK. (1943). Recovery of fibre numbers and diameters in the regeneration of nerve. Journal of Experimental Biology. Vol: 19 (14-44).

Gutmann L, and Medawar PB. (1942-b). The chemical inhibition of fibre regeneration and neuroma formation in peripheral nerves. Journal of Neuroscience Psychology. Vol: 5 (130-141).

Gutmann E, and Holubar J. (1951). Atrophy of nerve fibres in the central stump following nerve section and the possibilities of its prevention. Archives of International Studies of Neurology. Vol: 1 (489-518).

Harn SD, and Durham TM. (1990). Incidence of lingual nerve trauma and postinjection complications in conventional mandibular block anaesthesia. Journal of American Dental Association. Vol: 121 (519-523).

Hegtvedt AK, and Zuniga JR. (1990). Lingual nerve injury as a complication of rigid fixation of the sagittal ramus osteotomy: report of a case. Journal of Oral Maxillofacial Surgery. Vol: 48 (647-650).

Hiatt JL, and Gartner LP. (1982). Textbook of head and neck anatomy. Chapter 18 (279).

Hochwald DA, Davis WH, and Martinoff J. (1983). Modified distolingual splitting technique for removal of impacted mandibular third molars: incidence of postoperative sequelae. Oral Surgery. Vol: 56 (9-11).

Holland GR, and Robinson PP. (1990-a). The number and size of axons central and peripheral to inferior alveolar nerve injuries in the cat. Journal of Anatomy. Vol: 173 (129-137).

Holland GR, and Robinson PP. (1990-b). Cell counts in the trigeminal ganglion of the cat after inferior alveolar nerve injuries. Journal of anatomy. Vol: 171 (179-186).

Holland GR, and Robinson PP. (1992). Axon population in cat lingual and chorda tympani nerves. Journal of Dental Reseach. Vol: 71 (1468-1472).

Holmes W, and Young JZ. (1942). Nerve regeneration after immediate and delayed suture. Journal of Anatomy. Vol: 77 (63-106).

Horch KW, and Lisney SJW. (1981). On the number and nature of regenerating myelinated axons after lesions of cutaneous nerves in the cat. Journal of Physiology. Vol: 313 (275-286).

Howe GL. The extraction of teeth. Second edition. (1974). Chapter V (67-85).

Jenq CB, and Coggeshall RE. (1984). Effects of sciatic nerve regeneration on axonal population in tributary nerves. Brain Research. Vol: 295 (91-100).

Jenq CB, and Coggeshall RE. (1985-a). Numbers of regenerating axons in parent and tributary peripheral nerves in the rat. Brain Research. Vol: 326 (27-40)

Jenq CB, and Coggeshall RE. (1985-b). Long-term patterns of axon regeneration in the sciatic nerve and its tributaries. Brain Research. Vol: 345 (34-44).

Kiesselbach JE, and Chamberlain JG. (1984). Clinical and anatomic observations on the relationship of the lingual nerve to the mandibular third molar region. Journal of Oral Maxillofacial Surgery. Vol: 42 (565-567).

Kruger E, and Worthington P. (1981). Oral surgery in dental practice. Chapter X (205).

Lisney SJW. (1987). Functional aspects of the regeneration of unmyelinated axons in the rat saphenous nerve. Journal of the Neurological Sciences. Vol: 80 (289-298).

Lisney SJW. (1988). The proportion of sympathetic postganglionic and unmyelinated afferent axons in normal and regenerate cat sural nerves. Journal of the Autonomic Nervous System. Vol: 22 (151-157).

Lisney SJW. (1989). Regeneration of unmyelinated axons after injury of mammalian peripheral nerve. Quarterly Journal of Experimental Physiology. Vol: 74 (757-784).

Loughner BA, Larkin LH, and Mahan PE. (1990). Nerve entrapment in the lateral pterygoid muscle. Oral Surgery and Medical Oral Pathology. Vol: 69 (299-306).

Lubinska L. (1961). Demyelination and Remyelination in the proximal parts of regenerated nerve fibres. Journal of Comparative Neurology. Vol: 113 (275-280).

Lubinska L. (1982). Patterns of Wallerian degeneration of myelinated fibres in short and long peripheral stumps and in isolated segments of rat phrenic nerve. Interpretation of the role of axoplasmic flow of the trophic factor. Brain Research.

Vol: 233 (277).

Lundborg G. (1988). Nerve Injury and Repair. Churchill Livingstone.

Mason DA. (1988). Lingual nerve damage following lower third molar surgery. International Journal of Oral Surgery. Vol: 17 (290-294).

Mayhew TM, and Sharma AK. (1984). Sampling schemes for estimating nerve fibre size. I. Methods for nerve trunks of mixed fascicularity. Journal of Anatomy. Vol: 139 (45-58).

Mayhew TM. (1988). An efficient scheme for estimating fibre number from nerve cross sections: the fractionator. Journal of Anatomy. Vol: 157 (127-134).

Mayhew TM. (1990). Efficient and unbiased sampling of nerve fibres for estimating fibre number and size. Methods in Neurosciences. Vol: 3 (172-187)

Medinaceli L, and Seaber AV. (1989). Experimental nerve reconnection: importance of initial repair. Microsurgery. Vol: 10 (56-70).

Melville S, Sherburn TE, and Coggeshall RE. (1989). Preservation of sensory cells by placing stumps of transected nerve in an impermeable tube. Experimental Neurology. Vol: 105 (311-315).

Mozsary PG, Middleton RA, Szabo Z, and Machado L. (1982). Experimental evaluation of microsurgical repair of the lingual nerve. Journal of Oral Maxillofacial Surgery. Vol: 40 (329-331).

Mozsary PG, and Middleton RA. (1984). Microsurgical reconstruction of the lingual nerve. Journal of Oral Maxillofacial Surgery. Vol: 42 (415-420).

Myles LM, Gilmour JA, and Glasby MA. (1992). Effects of different methods of peripheral nerve repair on the number and distribution of muscle afferent neurons in rat dorsal root ganglion. Journal of Neurosurgery. Vol: 77 (457-462).

Ott L. (1977). An introduction to statistical methods and data analysis. Duxbury in press. Belmont, California.

Otto D, Unsicker K, and Grothe C. (1987). Pharmacological effects of nerve growth factor and fibroblast growth factor applied to the transectioned sciatic nerve on neuron death in adult rat dorsal root ganglia. Neuroscience Letters. Vol: 83 (156-160).

Peyronnard JM, Charron L, Lavoi J, and Messier JP. (1986). Differences in horseradish peroxidase labelling of sensory, motor and sympathetic neurons following chronic axotomy of the rat sural nerve. Brain Research. Vol: 364 (137-150).

Peyronnard JM, Charron L, Lavoie J, Messier JP, and Bergouignan FX. (1988). A comparative study of the effects of chronic axotomy, crush lesion and re-anastomosis of the rat sural nerve on horseradish peroxidase labelling of primary sensory neurones. Brain Research. Vol: 443 (295-309)

Pover CM, and Lisney SJW. (1988). An electrophysiological and histological study of myelinated axon regeneration after peripheral nerve injury and repair in the cat. Journal of the Neurological Sciences. Vol: 85 (281-291).

Ramon and Cajal. (1928). Degeneration and regeneration of the nervous system. Oxford University Press, London. Vol: 1.

Rezai RE, and Bayley NC. (1988). Lingual nerve damage: causative factors and management. Quintessence International. Vol: 19 (295-298).

Riediger F, Ehrenfeld M, and Cornelius CP. (1989). Micronerve surgery on the inferior alveolar and lingual nerve with special consideration for nerve replacement. In Microsurgical Tissue Transplantation, Eds Riediger D and Ehrenfeld M. Quintessence Publishing Co. Inc. (189-194).

Risling M, Remahl S, Hildebrand C, and Aldkogius H. (1980). Structural changes in kittens' ventral and dorsal roots L7 after early postnatal sciatic nerve transection. Experimental Neurology. Vol: 67 (265-279).

Risling M, Aldskogius H, Hildebrand C, and Remahl S. (1983). Effects of sciatic nerve resection on L7 spinal roots and dorsal root ganglia in adults cats. Experimental Neurology. Vol: 82 (568-580).

Robinson PP. (1989). The reinnervation of the tongue and salivary glands after lingual nerve injuries in cats. Brain Research. Vol: 483 (259-271).

Robinson PP, and Winkles PA. (1991). The number and distribution of fungiform papillae and taste buds after lingual nerve injuries in cats. Archives of Oral Biology. Vol:

36 (885-891).

Robinson PP. (1992). The effect of injury on the properties of afferent fibres in the lingual nerve. British Journal of Oral Maxillofacial Surgery. Vol: 30 (39-45).

Rood JP. (1983). Damage to inferior alveolar and lingual nerves during removal of impacted mandibular third molars. British Dental Journal. Vol: 154 (402-403).

Rud J. (1970). The split-bone technique for removal of impacted mandibular third molars. Journal of Oral Surgery. Vol: 28 (416-421).

Rud J. (1984). Reevaluation of the lingual split bone technique for removal of impacted mandibular third molars. Journal of Oral Maxillofacial Surgery. Vol: 42 (114-117).

Sanders FK, and Whitteridge. (1946). Conduction velocity and myelin thickness in regenerating nerve fibres. Journal of Physiology. Vol: 105 (152-174).

Schwartz LJ. (1973). Lingual anaesthesia following mandibular odontectomy. Journal of Oral Surgery. Vol: 31 (918-920).

Stacy GC. (1977). Lingual exposure during mandibular third molar surgery. International Journal of Oral Surgery. Vol: 6 (334-339).

Sunderland S. (1951). A classification of peripheral nerve injuries producing loss of function. Brain. Vol: 74 (491-516).

Sunderland S. (1978). Nerves and Nerve Injuries.

Churchill Livingstone.

Von Arx DP, and Simpson MT. (1989). The effect of dexamethasone on neurapraxia following third molar surgery. British Journal of Oral and Maxillofacial Surgery. Vol: 27 (477-480)

Waite PME. (1984). Rearrangement of neuronal responses in the trigeminal system of the rat following peripheral nerve section. Journal of Physiology. Vol: 352 (425-445).

Weinberg HJ, and Spencer PS. (1978). The fate of Schwann cells isolated from axonal contact. Journal of Neurocytology. Vol: 7 (555-569).

Weiss P, Edds MV, and Cavanaugh M. (1945). The effects of terminal connections on the calibre of nerve fibres. Anatomical Record. Vol: 92 (215-233).

Ygge J. (1989). Neuronal loss in lumbar dorsal ganglia after proximal compared to distal sciatic nerve resection: a quantitative study in the rat. Brain Research. Vol: 478 (193-195).

Zalewski AA. (1981). Regeneration of taste buds after reinnervation of a denervated tongue papilla by a normally non-gustatory nerve. The Journal of Comparative Neurology. Vol: 200 (309-314).